A LIGHT AND ELECTRON MICROSCOPIC STUDY OF CALCIFIED DENTAL TISSUES IN NORMAL HORSES

VOLUME 1

SERVET KILIC

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DECLARATION

This thesis is my own work and has not been presented to any other university other than the University of Edinburgh.

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This Thesis is dedicated to my father Mehmet and my mother Gulnaz.

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ABSTRACT

The structures of normal equine enamel, dentine and cement were examined in 50 teeth from 16 horses. Prior to examination these teeth were fractured, or sectioned by a lathe or diamond saw. Specimens were examined without further treatment or after acid etching or decalcification. Measurements of enamel thickness showed that in the transverse plane enamel thickness varied greatly throughout its folds, but that its thickness remained constant throughout the lengths of the tooth. Enamel was thickest in areas parallel to the long axis of the maxilla.

Three types of enamel were identified on scanning electron microscopic (SEM) examination and were termed equine types 1, 2 and 3 enamel according to the shapes of their prisms on transverse section and the amount and appearance of interprismatic enamel they contained. Equine type 1 enamel contained rows of prisms that were oval on cross section and were separated by thick interprismatic enamel plates. Equine type 1 enamel prisms were oriented at an angle of approximately 45° with both the amelodentinal junction and the occlusal surface. This enamel was found adjacent to the amelodentinal junction. Equine type 2 enamel contained prisms which ranged from circular to "horseshoe" shape on cross section and which were separated from each another by thin organic prism sheaths, with no interprismatic enamel present. Equine type 2 enamel prisms were oriented at a wide variety of oblique angles to the amelodentinal junction and the occlusal surface. This enamel was found adjacent to the amelocemental junction. Equine type 3 enamel was composed of round shaped prisms completely surrounded by large quantities of interprismatic material and this enamel was inconsistently present in a thin layer at the amelodentinal and amelocemental junctions. The largest component of peripheral enamel of upper teeth was type 1, compared to type 2 in the lower teeth. Incisor enamel contained increased proportion of type 2 enamel and unlike in cheek teeth, this consisted of bands of prisms that were aligned vertically, obliquely or horizontally to the occlusal surface. The diameters of types 1 and 2 enamel prisms were significantly greater in incisors than in cheek teeth.

Dentinal tubules were branched at the amelodentinal junction and extended towards the pulp cavity following straight vertical, oblique or "S" shaped courses depending on their origin in the teeth. The tubules of primary dentine were surrounded by a variable thickness of peritubular dentine and their position within the peritubular dentine varied from central to asymmetrical depending on their orientation in the teeth and the relationship between the origin of the dentinal tubules and the main pulp cavities. In primary dentine, the amount of intertubular dentine decreased and conversely the diameter of dentinal tubules and the amount of peritubular dentine increased, from the amelodentinal junction towards the junction of primary and secondary dentine. In regular secondary dentine peritubular dentine was absent, but the amounts of intertubular dentine and the density of dentinal tubules were increased compared to primary dentine. In irregular secondary dentine, the lumina of all dentinal tubules were obliterated. The number of dentinal tubules containing odontoblast processes increased from the amelodentinal junction to the junction of primary and secondary dentine. Dentinal tubules with double odontoblast processes were occasionally found. Regular secondary dentine contained many dentinal tubules without odontoblast processes. Peritubular dentine had a compact appearance and a smooth surface in untreated sections, but when etched it became rough and obliquely oriented canaliculae became apparent.

Equine cement contained lacunae which usually contained one, but occasionally two cementocytes. Significant differences were present between the diameters of cellular lacunae of infundibular and peripheral cement of upper and lower cheek teeth, but the ratio of lacunae: total cement volume and the number of lacunae/ unit area were non-significantly different between the same regions of equine cheek teeth cement. Two types of cemental defects were found, the first appearing as small round spaces along the amelocemental junction of both upper and lower cheek teeth. The second type was found only in infundibular cement and represented the site of the former vasculature. Peripheral cement was deposited both directly, i.e. on unresorbed or resorbed enamel surface or indirectly, where the cement was separated from enamel by a thin calcified organic layer.

SUMMARY

Gross and ultrastructural examinations of equine enamel, dentine and cement were undertaken on 46 cheek teeth and 4 incisors that were fractured, or sectioned with a lathe or diamond saw. Specimens were examined without treatment, after acid etching or decalcification utilising light, scanning electron and transmission electron microscopes at different magnifications. Equine cheek enamel was found to be extensively folded in the transverse plane and contained numerous pits and ridges on its dentinal and cemental interfaces, particularly the latter. The thickness of equine enamel varied greatly throughout its folds (in the transverse plane) and was thickest in areas where it was parallel to the long axis of the maxilla and mandible. However, enamel thickness remained constant in the longitudinal plane (throughout the length of the tooth).

This study defined three enamel types termed equine types 1, 2 and 3 enamels in equine teeth according to the transverse appearances of their prisms and the amount and appearance of interprismatic enamel they contained. Equine type 1 enamel contained alternating rows of oval shaped prisms and thick enamel plates and was found at the amelodentinal junction. Eq. type 2 enamel consisted of "keyhole" to "horseshoe" shaped prisms with little or no interprismatic enamel and was located at the amelocemental junction. Eq. type 3 enamel was composed of rounded prisms surrounded by large amounts of interprismatic enamel and was inconsistently present at both the amelodentinal and amelocemental junctions. Mandibular cheek teeth contain both types of enamel along all of its enamel folds.

In the cheek teeth, equine type 2 enamel prisms were oriented from oblique to near horizontal angles to the occlusal surface, whereas in incisor enamel they were oriented in multiple directions (decussated) including in the vertical, oblique and horizontal planes. Whilst prism decussation was only seen in the peripheral enamel of the cheek teeth, it was present throughout incisor enamel thus making incisors highly resistant to cracking.

The dentinal tubules extended from the amelodentinal junction towards the pulp in various orientation, depending on their sites in the teeth. The number of dentinal tubules/unit area and their diameters increased significantly from the amelodentinal junction towards the pulp cavities particularly in regular secondary dentine, but irregular secondary dentine contained no dentinal tubules. Dentinal tubules contained odontoblast processes that fully extended to the amelodentinal junction but odontoblasts were seldom found in regular secondary dentine, due to iatrogenic loss during specimen preparation.

In primary dentine the dentinal tubules were surrounded by large amounts of peritubular dentine that increased in diameter from the amelodentinal junction towards the junction of primary and secondary dentine. The site of the dentinal tubule within peritubular dentine varied at different sites. Peritubular dentine was surrounded by a thin layer of intertubular dentine which in acid etched sections gave dentine a honeycomb appearance.

The diameter of cement lacunae was greater in infundibular than peripheral cement of upper cheek teeth, which in turn was greater than that of lower cheek teeth cement. However no differences were found in lacunae numbers/unit area in the different

dentinal region. Two types of cemental hypoplasia were found in equine cheek teeth. The first, which was found at the amelodentinal junction of both peripheral and infundibular cement was termed junctional cemental hypoplasia and appeared as spaces varying from focal to long and narrow defects along the amelocemental junction with these defects surrounded by cement of normal appearance. The second type of cemental hypoplasia of cheek teeth termed central infundibular cemental hypoplasia was confined to the middle region of infundibular cement (in upper cheek teeth only) and the cement adjacent to the frequently large defects was very porous and contained large vascular channels. In recently erupted cheek teeth, the central infundibular cemental defects were filled with connective tissue. The size of the cemental defects, the relationships of such defects to the occlusal surface, the degree of porosity of cement surrounding these defects were believed to be important in the development of cement caries.

Peripheral cement was deposited both directly, i.e. on unresorbed or resorbed enamel surface or indirectly, where the cement was separated from enamel by a thin calcified layer. The surface of unresorbed enamel had a pitted appearance, with the bottoms of these pits formed by enamel prisms and the pit walls by interprismatic enamel. The cemental surface of resorbed enamel contained depressions of variable shapes and sizes. These depressions which are believed to be caused by the resorption of enamel by odontoclasts could be both focal and diffuse and were more marked on the cemental surface of infundibular as compared to peripheral enamel.

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GENERAL INTRODUCTION

The ancient Chinese literature shows that examination of the incisor teeth was used for ageing horses as early as 600 B.C. (Kertesz 1993). Galvayne (1912) described several anatomical features of incisor teeth to allow the ageing of horses and claimed that using his guidelines to perform ageing, that **"There is no record of me having made a single mistake".** However, critical studies including those of Walmsley (1993) and Richardson et al (1995) have shown that these dental features are of limited value in ageing horses, particularly those over 5 years old. Except for the ageing of horses, the veterinary literature of the 18th and 19th centuries contains little reference to equine dental studies. This is exemplified by a statement from an eminent 19th century veterinarian William Dick, the founder of this Veterinary School who in 1862 wrote **"of the disease of the teeth in the horse we know little"** (Dick 1862).

Dental related work is of great importance in general equine practice and a survey by the British Equine Veterinary Association (BEVA 1965) showed that 10% of time in equine practice involves dental related work including, dental rasping and chiselling, ageing of horses, assessing dental conformation and health during pre-purchase examinations and occasionally dental extractions. Despite the obvious importance of equine dental work, our knowledge of many areas of equine dental studies remain limited (Dixon 1993).

Progress has recently been made in some areas of equine dental studies, such as in clinical examination of dental disorders (Uhlinger 1988; Mueller 1991; Lane 1994), radiographic studies (Gibbs & Lane 1987; Dixon & Copeland 1993) and gross pathological studies (Baker 1974 & 1979) and Wafa (1988), with the latter studies

revealing that caries and periodontal diseases are major problems in the horse. Very limited histopathological studies have been performed on diseased equine teeth and this may not be surprising considering that the histological and ultrastructural characteristics of normal teeth are poorly understood. A thorough understanding of the normal appearance of these tissues is a prerequisite to performing similar examinations on diseased equine teeth.

Major advances in dental ultrastructural studies have become possible with the development of the electron microscope, particularly the scanning electron microscope (SEM) which allows examination of relatively thick dental specimens (Koenigswald & Clemens 1992). The dental structure and ultrastructure of species with brachyodont (short crowned) teeth, including humans, other primates and carnivores (dogs and cats), and of elodont species such as rabbits and mice have been well studied. This is in part due to the relatively small sizes of such teeth that allows them to be easily decalcified and examined. Additionally, in many studies these animals were used as experimental models for human dental disease.

Very many mammalian dental studies have been on the evolutionary and functional characteristics of teeth (Jones & Boyde 1974; Martin et al 1988; Fortelius 1985; Koenigswald & Clemens 1992; Kozawa 1992). Enamel with its specialised crystal and prism structures and also its great resistance to post mortem changes has dominated most evolutionary dental studies, although some studies, including those of Bradford (1967), Garberoglio & Brannstrom (1976) and Kierdorf & Kierdorf (1992) have shown that other dental structures, particularly peritubular dentine can be also of value for taxonomic studies.

Relatively few and limited ultrastructural studies on equine dental tissues have been reported. Quantitative calcium and phosphorus analyses of equine peritubular dentine were undertaken using electron probe microanalysis by Takuma (1960). Bradford (1963) examined some histochemical and biochemical characteristics of equine peritubular dentine, using it as a model for studies on human peritubular dentine. Jones & Boyde (1974) examined the deposition of coronal cement along the amelocemental junction in a small number of horses. The correlation between enamel ultrastructure and molarisation in various equid species was examined by Kozawa (1992). All of these studies have focused on limited dental characteristics and additionally most have utilised an unspecified or small number of samples and teeth. Koenigswald & Clemens (1992) noted that such studies describe only a limited part of a very complex structure. Additionally, the structure of dental tissues may vary at different sites and thus the use of small numbers of samples from a tooth may not give representative results.

Although the development of high resolution electron microscopes have facilitated the study of the smallest structural units of dental tissues in great detail, the use of such microscopes has tempted researchers to focus their attention on only the smallest structures and thus to lose sight of the overall structure and of the interrelationships of the dental tissues (Koenigswald & Clemens 1992). In this particular study we aimed to overcome such a temptation by utilising different types of microscopes, i.e. light, scanning electron and transmission electron microscopes and also by using a wide range of magnifications on large numbers of specimens from representative areas of a large number of equine teeth.

The aim of this study is to describe the normal gross, histological and ultrastructure of the calcified dental tissues, i.e. enamel, dentine and cement of equine teeth. By defining the normal equine dental structure, further studies on structural changes in equine dental disease can then be more rationally pursued.

CHAPTER 2. REVIEW OF THE LITERATURE

2.1. Evolution of equine teeth

The modern horse (*Equus caballus*) evolved from a small leaf browsing animal called *Hyracotherium* (which means "rabbit like animal"). *Hyracotherium* which is also known as *Eohippus* or "Dawn Horse" migrated from North America to Europe through the Greenland Bridge, which then connected Alaska and Asia. In the early Neogene era with the spread of grassland due to climatic changes, a horse lineage with stocky characteristics and reduced number of toes evolved which ate grass (Bennet 1992). This lineage evolved a highly efficient digestive system for utilising grass, including the development of caecal digestion and evolutionary development of its teeth allowed efficient mastication of grass (Bennet 1992).

Hyracotherium's incisors were shovel shaped with a flat terminus and its canine teeth were short and flattened from side to side (Bennet 1992). *Hyracotherium* plucked or tore its food with its lips rather than with its incisors. In contrast, its successor developed specialised incisor teeth for efficient prehension of food and cheek teeth for chopping and crushing.

Hyracotherium had four premolars and three molars in each jaw. With evolution, the first premolar (wolf teeth) became smaller, separated from the other premolars and did not come into direct contact with the opposing tooth. The caudal premolars (PM2-4) and the three molars of each jaw quadrant remained in full apposition. The cusps of these teeth became aligned to allow efficient side to side mastication. The upper cheek teeth became broader and squarer as compared to the lower teeth (Bennet 1992).

Over 5 billion years of evolution, these teeth underwent continuous change. Because grass contains tiny particles of silica, chewing grass causes much wear of teeth and the simple short crowned (brachyodont) teeth of *Hyracotherium* would quickly wear out on such a diet. Consequently, these cheek teeth evolved into the high crowned (hypsodont) teeth of the modern horse that have prolonged eruption. The most significant dental evolution however was the molarisation of the cheek teeth. Molars are described as hypsodont teeth with cusps, enamel foldings and thick coronal cement (Kozawa 1992). Another evolutionary adaptation to a herbivorous diet was the development of teeth with bands of material with different degrees of hardness that wore at different rates. This feature produced a self sharpening occlusal surface that facilitates efficient grinding of a fibrous diet (Bennet 1992).

2.2. EMBRYOLOGY OF TEETH

Dental development (dentogenesis) involves several complex biological processes including epithelial-mesenchymal interaction, growth, remodelling and calcification of tissues until a tooth is fully developed (Warshawsky 1983; Fortelius 1985; Ten Cate 1994). Although the precise mechanisms for determining the final shape of teeth are still unknown, two theories have been suggested. The first is the "gradient" theory which assumes that dental shape is controlled by extrinsic factors such as compression of developing germs by adjacent structures such as the mandible or maxilla. The second, or so called "cell lineage" theory is that tooth shape is primarily determined by intrinsic (ontogenetic) factors in the developing tooth germs (Kollar & Lumsden 1979; Fortelius 1985).

During dental development, the tooth germ undergoes a series of distinct, consecutive events termed; the initiating, morphogenetic and cytodifferentiative

phases. The morphogenetic phase is the development of the dental papilla and folding of the basal membrane that later becomes the amelodentinal junction (Fortelius 1985). The cytodifferentiative (histodifferentiative) phase of dental development refers to the developmental stage during which the cells of the enamel organ transform morphologically and functionally into specialised dental cells (Ten Cate 1994). The initiative, morphogenetic and cytodifferentiative phases occur in all types of mammalian dentition, i.e. brachyodont, hypsodont and elodont (Kollar & Lumsden 1979), however, their timing and termination vary i.e. hypsodont teeth have a delayed termination of the morphogenetic and cytodifferentiative stages, whilst in elodont teeth, these stages proceed at the apical dental region throughout all of the animal's life. The presence of similar initiative, morphodifferentiative and cytodifferentiative events in all mammals indicate that dental development is controlled, at least partially, by similar genetic factors (Fortelius 1985).

The first sign of tooth formation is the development of a horseshoe shaped epithelial thickening along the lateral margin of the primitive oral cavity. This epithelial thickening, termed the primary epithelial band, invaginates into the underlying mesenchymal tissue to form two distinct ridges, one in front of the vestibular lamina and one behind the dental lamina. The vestibular lamina separates the lip from the gum and contributes to the formations of the cheeks and lips (Warshawsky 1983; Ferguson 1990; Ten Cate 1994).

As it extends rostrally along the gingival line, the dental lamina produces a series of epithelial swellings, called tooth buds along its buccal margin. This stage is known as the "bud" stage of tooth development (fig 1). At this stage, a mesenchymal cellular proliferation starts to develop beneath the tooth bud. With

continuing proliferation, these mecenchymal cells invaginate into the ectodermal tooth bud which now develops into an inverted cap shaped structure called the enamel organ and this is called the "cap" stage of dental development (fig 1).

All deciduous teeth and the permanent molars develop from the enamel organ of the dental laminae. However, permanent incisors, canines and premolars are formed from separate enamel organs that are derived from labial extensions of the dental laminae of the deciduous teeth. In the equine foetus, the first deciduous tooth buds are recognised at 4 months of gestation, and the first permanent tooth buds at 9.5 months of gestation (Baker 1985a).

With formation of the enamel organ, the cells of the underlying ectomesenchyme continue to proliferate within the concave aspect of the enamel organ, as well as over the peripheral surface of this structure. This mesenchymal tissue now becomes divided into two discrete parts, termed the dental papilla and the dental follicle (dental sac) (fig 1).

The dental papilla fills the concave aspect of the enamel organ and is responsible for dentine and pulp formation (Brescia 1966; Berkovitz & Moxham 1981). At the early cap stage, a distinct feature of the dental papilla is the presence of collagenous fibrils oriented radially between the extracellular spaces of papillary cells (Ten Cate 1994).

The dental follicle encloses the convex face of the enamel organ and contains many collagenous fibrils that are scattered randomly between fibroblasts. (Ten Cate 1994). At this stage, the dental follicle develops into three distinct layers, called the inner vascular, loose connective tissue and outer vascular mesenchymal layers (Jones 1981). The enamel organ and dental papilla are surrounded and protected by

the dental follicle until tooth eruption. Traumatic damage or infection of the follicular layer can cause abnormal tooth formation (Ferguson 1990).

The enamel organ, dental papilla and dental follicle are together termed the tooth germ, with each germ responsible for an individual tooth (Ferguson 1990). In the developing maxillary and mandibular arcades of a 120 day equine foetus, three tooth germs were observed in each quadrant, giving a total of 12 tooth germs. The second deciduous premolar germs were larger than those of the third and fourth deciduous premolar teeth, which suggests that in horses the second premolar tooth develops first (Baker 1985a).

The enamel organ in the tooth germ proliferates further and in the brachyodont dentition assumes a bell shape, which is termed the "bell" stage of tooth development. At this stage, the concavity of the enamel organ increases while the cells of the dental papilla invaginate further into enamel organ (fig 1). The cells at the cervical junction (loop) of internal and external enamel epithelia grow down over the dental papilla.

Most cytodifferantiative events in the tooth germs occur during the transitional period between the cap and bell stages. The first sign of the cytodifferentiative phase is the differentiation of the uppermost epithelial cells of the enamel organ into the internal and external enamel epithelia. The cells lining the concave aspect of the enamel organ become the internal enamel epithelium and the cells lining the convex aspect of the enamel organ form the external enamel epithelium (Fortelius 1985). At the early bell stage, three distinct regions, i.e. the stellate reticulum, and external and internal enamel epithelia are differentiable. The stratum intermedium develops over the internal enamel epithelium towards the late

bell stage of tooth development. At this stage, the dental laminae of permanent teeth emerge at the labial aspects of the deciduous teeth germs. Additionally the internal enamel epithelial cells differentiate into ameloblasts and the undifferentiated ectomesenchyme cells differentiate into odontoblasts and these cells will later respectively, produce enamel and dentine (Berkovitz & Moxham 1981) (fig 1).

2.2.1 External enamel epithelium

The cells of the external enamel epithelium differentiate from the outer epithelial cells of the enamel organ. They are short and columnar shaped with large centrally located nuclei. They maintain this shape and remain attached to each other and to the stellate reticulum by specialised intercellular structures termed desmosomes (also known as attachment plaques or macula adherents), and to the basement membrane by hemidesmosomes. The external enamel epithelium is believed to control the exchange of substances between the enamel organ and the dental follicle and thus maintains the integrity of the enamel organ during dental formation (Berkovitz & Moxham 1981; Ten Cate 1994).

2.2.2. Basal membrane

The basal membrane marks the site of the amelodentinal junction whose orientation determines the shape of a developing tooth (Pannese 1962b; Fortelius 1985). The basal membrane surface contains fibronectin, a high molecular weight glycoprotein, which first attracts the preodontoblastic cells to align along the basal membrane. Tissue culture experiments have shown that the basal membrane alone can induce preodontoblast cells to differentiate into odontoblasts (Ten Cate 1994).

2.2.3. Stellate reticulum

At the early cap stage, the cells located between the external and internal enamel epithelia secrete various mucopolysaccaride molecules, including glycosaminoglycans with highly hydrophilic characteristics that expand their intercellular spaces. This intercellular expansion reaches maximum levels at the late cap stage. As a result, these cells develop stellate (star-like) shapes with long cytoplasmic extensions and large spaces between cells (Warshawsky 1983; Ferguson 1990). This tissue is termed the stellate reticulum and, has nutritive and mechanical functions in dental development. Its nutritive function is due to the presence of its abundant glycosaminoglycans (Berkovitz & Moxham 1981). Mechanically, the stellate reticulum stabilises the position of the internal enamel epithelium by neutralising the pressure of the dental papilla within the enamel organ. Thus, a change in content of either the stellate reticulum or the dental papilla can alter the position of both the internal enamel epithelium and the basal membrane and thus the final shape of the tooth (Berkovitz & Moxham 1981).

2.2.4. Temporary structures of the enamel organ

In addition to the development of external and internal enamel epithelia, stratum intermedium and stellate reticulum during the late cap stage, the enamel organ also develops two other structures called the enamel knot and the enamel cord or septum (Ferguson 1990; Ten Cate 1994). The enamel knot is a localised thickening of the internal enamel epithelium at the centre of the tooth germ. A strand of cells extending from the enamel knot to the external enamel epithelium is termed the enamel cord. These structures may function to determine the initial positioning of the first tooth cusp (Ten Cate 1994).

2.2.5. Stratum intermedium

During the mid to late bell stage, a layer of a few cells in thickness, called the stratum intermedium develops over the internal enamel epithelium of the enamel organ. The stratum intermedium is surrounded externally by the stellate reticulum. The cells of the stratum intermedium are connected to each other and to the internal enamel epithelial cells by desmosomes. The stratum intermedium cells synthesise proteins that control transportation of fluid, mineral, enzymes and growth factors between the internal enamel epithelial cells and the stellate reticulum (Brescia 1966; Ferguson 1990).

2.2.6. Dentine and enamel deposition

Dentine and enamel deposition begins at the late bell stage of dental development due to a series of reciprocal epithelial-mesenchymal interactions.

2.2.7. Epithelial-mesenchymal interactions

At the late bell stage, the short, columnar shaped cells at the cervical region of the internal enamel epithelium divide rapidly to accommodate the proliferating dental papilla. The dental papilla contains small undifferentiated ectomesenchymal cells with a centrally located nuclei that are present in a relatively structureless ground substance that contains a few collagen fibrils. At this stage of dental development, an acellular zone separates the dental papillary cells from the internal enamel epithelium. After cell division ceases, the cells of the internal dental epithelium develop into tall columnar cells with large, proximally located nuclei.

This change induces alterations at the molecular level in the underlying dental papilla whose uppermost cells now move toward the cell-free zone and align along the basal membrane in a single layer. These cells now rapidly enlarge, first

becoming preodontoblasts and then odontoblasts as their cytoplasm increases in volume and develops large amounts of rough endoplasmic reticulum and Golgi complexes. Recent studies on mouse teeth indicate that the ectomesenchymal cells of the dental papilla must undergo several cell differentiation stages before they attain the capacity to respond to epithelial induction (Ten Cate 1994). Immediately after the odontoblasts mature, the first dentine layer is laid down along the basal membrane. With mineralisation of this layer, the basal membrane disintegrates. These changes reciprocally induce the overlying internal enamel epithelial cells to differentiate into ameloblasts which now begin to produce enamel (Ferguson 1990).

2.2.8. Ameloblasts

Ameloblasts have large, proximally located nuclei and contain large amounts of rough endoplasmic reticulum, Golgi complexes, ribosomes, microfilaments and microtubules. They are closely aligned and attached together by specialised structures termed the junctional complex. Fine, actin containing filaments extend from the junctional complex toward the cytoplasm to form the distal and proximal terminal webs. The junctional complex can becomes tight or loose to regulate movement of materials to and from enamel (Pannese 1962b; Eisenmann 1994). After the initial deposition of a structureless enamel layer, the ameloblasts migrate away from the dentine surface and form a projection termed Tomes process at their distal surface.

Although the cytoplasm of the ameloblast cell bodies and Tomes' processes are similar, they are distinctly separated by the distal terminal web and junctional complex (Eisenmann 1994). Secretions from the proximal aspect of Tomes' process (close to the junctional complex) form interprismatic enamel, but secretions from

the surface of Tomes' processes form the enamel prisms. Electron microscopic (EM) studies have shown that ameloblasts release their protein and glycoprotein secretions in granular forms into the extracellular spaces (Warshawsky 1983). Early in enamel formation (amelogenesis), collagenous filaments can be observed adjacent to the irregularly shaped distal ends of ameloblasts and may function to join the enamel and dentine together (Ronnholm 1962).

2.2.9. Odontoblasts

While major cytodifferentiative events are occurring in the enamel organ, the small undifferentiated cells of ectomesenchyme differentiate into odontoblasts. Odontoblasts, like ameloblasts and cementoblasts are end cells, meaning that they cannot further differentiate into other cell types. While dentine deposition continues, the basal aspects of odontoblasts gradually become thinner and form cytoplasmic extensions termed odontoblast processes which are demarcated from the cell bodies by terminal webs (Baker 1979; Eisenmann 1994). Odontoblast processes contain no cytoplasmic organelles except a few mitochondria at their proximal aspect, but they contain cytoskeletal structures including dense rod-shaped bodies, microvesicles, coated vesicles, filaments and microtubules. These microtubules may be involved in the intracellular circulation of cytoplasm and the coated vesicles may play a role in collagen secretion (Jessen 1967).

Light microscopic (LM) examinations have revealed two distinct stages of odonoblast activity, termed the secretory and resting stages, however, EM examinations have shown the presence of a transitional period between these two stages. At the secretory stage, odontoblast are circa 35 μm in length and have a swollen appearance. They contain copious amounts of cytoplasm and endoplasmic

reticulum, Golgi apparatus and mitochondria and they have large nuclei with several nucleoli. In the transitional stage, their cell bodies gradually lose their cytoplasm and their intracelullar organelles condense around the nucleus. At the resting stage, they appear as small shrunken cells in LM examination. The nuclei of resting stage odontoblasts are located closer to the apical ends of cells than those of odontoblasts in active and transition stages (Ten Cate 1994). Although they may be morphologically in the resting phase, odontoblasts always remain capable of synthesising dentine throughout their lives if appropriately stimulated (Osborn 1981; Ten Cate 1994).

In human teeth the shape of odontoblasts vary at different locations, i.e. they are columnar shaped in coronal dentine, cuboidal in the mid tooth region and flattened in apical dentine (Torneck 1994). Measurements of dentinal tubule numbers show that the number of odontoblasts remains constant during the formation of primary dentine and in the early stages of secondary dentine formation. In the later stages of secondary dentine formation, their numbers decrease as some become trapped in their secretions due to overcrowding and some may disintegrate due to ageing (Berkovitz & Moxham 1981; Jones 1990).

Odontoblast cell bodies are attached to each other by structures termed the junctional complex which include desmosomes (zonulae adherents), tight junctions (zonulae occludentes) and gap junctions (zonulae communicants) whose locations vary according to odontoblast activity. Although the precise functions of junctional complexes remain unclear, they may control the passage of extracellular material, particularly of calcium and phosphorus ions. The presence of gap junctions between

odontoblast indicates that free interchanges of ions and small molecules occurs between these cells (Torneck 1994).

2.2.10. Mineralisation of enamel and dentine

Mineralisation begins at the tips of single cusped teeth and then progresses over the cusp and slopes down towards the amelodentinal junction. In multicusped teeth, mineralisation starts independently at each cusp tip and then merges as calcification progresses down along the amelodentinal junction (Berkovitz & Moxham 1981). As dentine and enamel deposition continues, odontoblasts and ameloblasts move in opposite directions to avoid being entrapped in their own secretions.

The development of both enamel and dentine occurs in two consecutive phases, the secretion of extracellular matrix that is followed by its mineralisation. Ameloblasts secrete a matrix containing proteins which mineralises immediately after it is secreted, thus no equivalent of predentine or osteoid tissue exists in enamel development (Eisenmann 1994). Microradiographic and chemical studies in the rat, dog and cattle have shown that enamel mineralisation has four stages. These stages are consecutively i. secretion of a soft, translucent, partially mineralised enamel, ii. withdrawal of matrix from this soft tissue that provides fluid filled spaces for further mineral deposition iii. secondary mineralisation of the increasingly rigid enamel, iv. emergence of hard, translucent, mature enamel (Suga 1979; Eisenmann 1994).

Radiography has shown that the calcification of equine deciduous teeth buds begins at 120 days of fetal life and is completed by 240 days, while calcification of the first permanent tooth begins 6 months later (Baker 1979; Baker 1985a).

2.2.11. Involution of the enamel organ

Involution of the enamel organ starts in the late bell stage of dental development and as noted, is followed by the deposition of the initial dentinal layer. With enamel organ involution, the internal enamel epithelial cells become closer to the vascular capillaries of the dental follicle that surround the external enamel epithelium.

Polarised LM, EM and histochemical studies have shown that involution starts at the external enamel epithelial layer and proceeds toward the stellate reticulum and stratum intermedium (Pannese 1962a). Following disappearance of the nuclei, the cytoplasm becomes filled with packages of tonofilaments, mitochondria and endoplasmic reticulum. These cells are now transformed into plate shaped structures which are embedded in less dense amorphous material. The involution of the stellate reticular cells is so rapid, that the intermediate stages of involution are rarely found. The involution of the cells of the stellate reticulum is similar to the cytomorphosis of the stratified squamous epithelium of the cornea (Pannese 1962a).

2.2.12. Fate of the dental lamina

As dental development proceeds at the late bell stage, the dental lamina gradually shrinks and then breaks up into discrete islands of epithelial cells which normally disappear (Brescia 1966; Warshawsky 1983). However, these epithelial cell islands may occasionally persist and are termed "the glands of Serres" by Berkovitz & Moxham (1981) or "epithelial pearls of Serres" by Boyde (1990). Their presence may result in delayed dental eruption or even in the development of enamel tumours (ameloblastomas) (Warshawsky 1983; Ten Cate 1994). Once the

dental lamina has degenerated, the tooth germ retain a connection with the overlying oral epithelium through a fibrous band that is termed the gubernacular cord that develops between the dental follicle and oral epithelium (Boyde 1990).

2.2.13. Vascularisation of the tooth germ

Vascularisation begins at the periphery of the tooth germs at the early cap stage and blood vessels continue to ramify into the dental follicle and extend into the dental papilla until the late cap stage, reaching a peak at the late bell stage (the onset of dentinogenesis) (Ten Cate 1994). Until this stage, the internal enamel epithelium is supplied by small ectomesenchymal capillaries. When dentinal mineralisation starts the connection between the internal enamel epithelium and the dental papilla is completely lost and ameloblasts now utilise stored glycogen and also obtain nutrition from the stratum intermedium and stellate reticulum (Ferguson 1990).

2.2.14. Innervation of dental germs

Nerve fibres approach the developing dental germs during the transition of the tooth germ from the bud to the cap stage. Histological studies have revealed that these fibres are purely sensory with no autonomic nerves detected at this stage. During the cap and bell stages, these sensory nerve fibres ramify into the dental follicle to form a neural plexus, but nerves have not been identified in the dental papilla before dentine formation (dentinogenesis), i.e. the late bell stage of dental development. These sensory nerve fibres appear in dental germs earlier than blood vessels which as previously noted reach the dental germs at the late cap stage and are accompanied by sympathetic nerve fibres (Ten Cate 1994).

2.2.15. Hertwig's epithelial root sheath

After crown formation is completed in brachyodont teeth, the external and internal enamel epithelial cells at the cervical region proliferate down over the dental papilla as a double layer of cells called Hertwig's epithelial root sheath. This epithelial sheath induces the underlying mesenchymal cells to differentiate into odontoblasts, which in brachyodonts, later produce root dentine. When the mineralisation of root dentine begins, Hertwig's epithelial root sheath is disintegrated by the proliferating overlying connective tissue. However, some epithelial sheath cells may occasionally persist to form structures termed "the epithelial cell rests of Malassez" adjacent to the cemento-dentinal junction. Although normally inactive, in humans with periodontal inflammation these cells can occasionally form dental cysts (Ten Cate 1994).

2.2.16. Cementogenesis

After the disintegration of Hertwig's epithelial root sheath, the dental follicular cells come into direct contact with dentine at an area termed the cemento-dentinal junction. In brachyodont teeth, this junction is not as distinct as the amelodentinal junction. Interaction between these two tissues now induces cells of the dental follicle to convert into cemental forming cells, i.e. cementoblasts (Brescia 1966; Warshawsky 1983).

In equine teeth, cement is laid down on the large enamel surface of the crown and on the dentinal surface of the small apical region, starting respectively at circa 210 and 280 days of fetal life (Baker 1985a). Cemental deposition at the apex is similar to that in brachyodont teeth. However, coronal cemental deposition, which

does not occur in brachyodont teeth, is followed by disintegration of the reduced enamel epithelial layer, possibly by osteoclasts (Jones & Boyde 1974).

Additionally, in the upper cheek teeth and in all the incisors, cement is also laid down on the occlusal surface and deposition proceeds toward the depths of the infundibula by cementoblasts nourished by capillaries of the dental follicle (Baker 1985a; Barker et al 1993). The presence of a patent central vascular channel in the infundibular cement of many horses represents the sites of these embryological blood vessels called the "gubernacular cord" by Baker (1979). Cement initially has a sponge-like appearances and reaches maturation in two consecutive stages, i.e. secretion of organic matrix and the mineralisation of the matrix (Baker 1974; Baker 1985a).

2.3. GROSS ANATOMY

2.3.1. Dental classification

Mammalian teeth are divided into two main groupings termed elodont (continuously growing) and anelodont (with a limited growth period) teeth (Kertesz 1993). Anelodont teeth can be subdivided into hypsodont (long crowned) and brachyodont (short crowned) teeth according to their crown: root ratio. Omnivores, carnivores, and non-grazing herbivores have brachyodont (short crowned) teeth, but all grazing and browsing herbivores, including horses, have hypsodont teeth. Brachyodont teeth have a distinct neck between the crown and root, a feature not present in hypsodont teeth. At eruption, hypsodont teeth have no true roots and in this study the term root specifically refers to the apical area which is enamel free (DeLahunta & Habel 1986; Miles & Grigson 1990).

2.3.2. Dental functions

Adult mammalian mouths have four types of teeth, termed incisors, canines, premolars and molars, in a rostro-caudal order. Each type of tooth has certain morphological characteristics and specific functions which include the prehension and preparation of food for digestion. Incisor teeth, especially in carnivores, are specialised for the prehension and cutting of food. The canine teeth are for defence, for capture of prey by carnivores, and are used for excavation by animal with tusks. The cheek teeth function as grinders for mastication (St. Clair 1975; Kertesz 1993). In addition, teeth are used for grooming and serve in sexual dimorphism and mate selection (Amand & Tinkelman 1985; Kertesz 1993). The horse is equipped with an efficient dental apparatus for grazing and grinding food. Their prolonged eruption and high crown characteristics compensate for the high rate of wear, maintaining an efficient dental arcade throughout the horse life.

2.3.3. Equine dental formula

All domestic animals including the horse are diphyodonts, i.e. have two sets of teeth, termed temporary (deciduous, primary or milk) and permanent (secondary) teeth (St. Clair 1975). In foals the deciduous teeth which consist of incisors and premolars are erupted at birth or erupt shortly afterwards. The deciduous teeth are replaced by the larger permanent teeth, but in the horse the transverse (cross sectional) area of the deciduous cheek teeth is similar to that of the adult, in contrast to brachyodont teeth (Dixon & Copeland 1993). In the current work, the term "transverse" refers to a plane perpendicular to the long axis of body or part (Nomina Anatomica Veterinaria 1983) and will be used for example, to refer to a plane of section of the entire tooth, of dental structures such as dentinal tubules or enamel

prisms and to ultrastructures such as enamel crystals. Macroscopically, deciduous incisors are whiter and contain wider and shallower infundibula than their permanent successors, which usually erupt on their lingual aspect. As previously noted, the eruption of deciduous and permanent teeth can be used to estimate the age of horses up to 5 years old with a reasonable degree of accuracy (Walmsley 1993; Richardson et al 1994). The dental formula of deciduous and permanent teeth in horses are:

Deciduous teeth: 2 (Di 3/3, Dc 0/0, Dm 3/3)= 24

Permanent teeth: 2 (I 3/3, C 1/1, PM 3/3 or 4/3, M 3/3)= 40 or 42 (St. Clair 1975):

2.3.4. Incisors

Horses have 12 incisors in total, 6 in each arcade which are termed central, intermediate (middle) and corner incisors and are shown numerically as I1(first), I2 (second), I3 (third). The upper incisor teeth are embedded in the incisive (premaxilla) bone and the lower incisors in the rostral mandible. These teeth are curved convexly on their labial aspect and uniformly taper in from the occlusal surface toward the apex. A fully developed incisor arcade in a young adult horse has an almost semicircular appearance, which gradually becomes more shallow with age due to alteration of teeth shape caused by progressive wear (St. Clair 1975; Baker 1985a).

In addition to their eruption times, equine incisor teeth contain certain macroscopic features that are related to wear, which have also been traditionally utilised for estimating age (Goody 1983). These structures are the incisal cup (cup), enamel spot (mark), dental star, hooks, presence and size of Galvayne's groove on the upper corner incisors, the occlusal angle between the upper and lower incisors and the shape of the occlusal surface of lower incisors. In this regard, the disappearances of the cup, the occlusal angle between the upper and lower incisors, the shape of

occlusal surfaces of lower incisors, and Galvayne's groove are considered as relatively reliable indicators of age whilst the disappearance of the mark and the presence of a notch in the caudal upper incisor are unreliable features of age (Walmsley 1993; Richardson et al 1994). Variations in incisal teeth appearance can also be due to individual variation, environmental conditions, eruption times, mineralisation rates, depth of enamel infundibulum, amount of infundibular cement and the presence of certain stereotypic behaviours such as crib-biting and windsucking (Eisenmenger & Zetner 1985; Mueller 1991; Richardson et al 1994).

2.3.4.1. Shape of the incisor occlusal surface

The occlusal surface of incisors are elliptical in recently erupted incisors, but with wear, they successively become round, triangular and then oval in shape. These changes are more apparent in the central (I1) and intermediate (I2) than in the corner (I3) incisors (St. Clair 1975; Richardson et al 1994).

2.3.4.2. Incisal cup

The infundibulum present in incisor teeth is termed the incisal cup. This funnel like structure is almost circular in shape and circa 10 mm deep when the tooth first erupts. This infundibulum is usually incompletely filled with cement and consequently become filled with decomposing food material and therefore looks dark (Walmsley 1993). Galvayne (1912) claimed that in the mandibular arcade, the cups disappear by 6 years of age in central, 7 years in intermediate and 8 years in the corner incisors. However, critical studies including those of Walmsley (1993) and Richardson et al (1994) have shown that cups may disappear 1 or 2 years earlier than traditionally expected.

2.3.4.3. Enamel spot

When the infundibulum is worn away, it leaves behind a small, ring of infundibular enamel located on the lingual aspect of the tooth which is called the enamel spot (enamel ring or mark) (Baker 1985a; Walmsley 1993). Due to the slower wear of enamel as compared to dentine, the enamel spot is elevated above the occlusal surface. Many authors including Goody (1983), Dyce et al (1987) and Mueller (1991) stated that the enamel spot disappears by 15 years of age. However, Richardson et al (1994) found these structures absent in a 9 year old horse, yet present in a 20 year old horse.

2.3.4.4. Dental star

The dental star represents exposure of secondary dentine within a former pulp cavity on the occlusal surface of incisor teeth. It appears sequentially in the central, middle and corner incisors at between 6-14 years of age. It first appears as a dark yellow transverse line on the labial aspect of the cup, then with further tooth wear it gradually becomes oval in shape and moves toward the centre of occlusal surface. It has a round appearance in horses between 10-15 years of age (Goody 1983).

2.3.4.5. Galvayne's groove

Galvayne's groove is a longitudinal depression that appears on the labial aspect of the upper corner (I1) incisors and has also been considered as an useful criterion for ageing horses by Galvayne (1912) who reported that it first appears at the gingival margin at 10 years of age, extends about half way down the clinical crown at 15 years, comes into wear at 20 years of age and totally disappears by 30 years of age. However, Richardson et al (1994) observed that Galvayne's groove

extended three quarters of the length of the clinical crown in an 11 year old horse, yet extended down only half of the length of the clinical crown in a 20 year old horse.

2.3.4.6. Hook (Notch)

A hook, a localised overgrowth, is often recognised at the caudolabial aspect of the occlusal surface of the upper corner incisor (I3) at any time after 6 years of age (DeLahunta & Habel 1986; Richardson et al 1994). It is caused by incomplete occlusal contact between the upper corner incisor with its opposing lower tooth and is sometimes called a "seven year notch" because it was traditionally believed to appear at seven years of age (Goody 1983; DeLahunta & Habel 1986).

2.3.5. Canine teeth

Male horses normally have four canine teeth; two maxillary and two mandibular, that erupt at 4-6 years of age in the interdental space (St. Clair 1975; Mueller 1991). They are simple teeth (i.e. contain no coronal cement or enamel foldings) and are pointed, with a caudal facing curve. There is no occlusal contact between the upper and lower canines, with the upper ones being located more rostrally (Baker 1985a). Canine teeth are absent or are rudimentary in female horses (St. Clair 1975). Colyer (1906) found rudimentary canine teeth present in 48 out of 173 mares (27.8 %).

2.3.6. Cheek teeth

As the last three premolars (PM2, 3 & 4) and the 3 molars (M1, 2 & 3) are very similar in structure and appearance, they are all commonly termed as cheek teeth. An adult equine mouth normally contains 24 cheek teeth, forming four rows of 6 teeth that are accommodated in the maxillary and mandibular bones. Equine cheek teeth contain multiple cusps as shown in figs 2 & 3 (Miles & Grigson 1990).

The cheek teeth act as grinders, utilising a side to side movement, that is combined with a slight rostro-caudal movement of temporomandibular joint. On transverse section, equine cheek teeth are rectangular shaped, except the first and last, i.e. PM2 and M3 which are somewhat triangular shaped (St. Clair 1975). Cheek teeth possess long crowns, most of which is unerupted reserve crown that is embedded in the alveolus and gingiva. At eruption, cheek teeth have no or relatively short true roots (enamel free), but these develop and elongate with age. The upper cheek teeth have three roots (two small lateral and a larger medial) and the lower cheek teeth (except M3 which has three roots) possess two roots, one rostral and one caudal (St. Clair 1975; Baker 1985a). One or both of the upper PM1 and less commonly, the lower PM1 can also be present as the small, vestigial "wolf teeth" with a reported incidence of these teeth of 24.4% in females and 14.9% in males by Colyer (1906), and in both sexes, of 13% by Baker (1979) and of 31.9% By Wafa (1988).

Dental eruption proceeds throughout the life of the equine tooth and normally growth rate corresponds with tooth wear (attrition). Dental wear in horses and zebras has been calculated as 2-3 mm per year (Baker 1985a; Dyce et al 1987). Therefore, since all permanent teeth come into wear by 5 years of age, a horse's teeth should be fully worn by 30- 35 years of age (Baker 1985a).

Both the maxillary and mandibular cheek teeth form a slightly curved row, with their concavity toward the buccal and lingual aspect respectively (St. Clair 1975). The reserve crowns of the upper and lower first cheek teeth (PM2) are directed slightly rostrally, those of the third premolar are perpendicular and those of three caudal cheek teeth (molars) are curved caudally. The cheek teeth are in very

close contact at the occlusal surface even though their reserve crowns and roots greatly diverge. Pressure from the caudally facing first cheek teeth and the rostrally facing caudal cheek teeth compress the 6 cheek teeth together and along with the continuing deposition of coronal cement, maintains this tight occlusal contact throughout life in the normal horse (Hofmeyr 1960; Baker 1985a; Dixon and Copeland 1993).

The caudal 3-4 cheek teeth are embedded in the rostral and caudal maxillary sinuses and in the young horse their large reserve crowns occupy a large part of these sinuses. With age and eruption of reserve crowns, the residual sinus cavities increase in volume. This intimate relation between the cheek teeth and sinuses can allow periapical infections of the caudal cheek teeth to cause maxillary sinus empyema.

Equine cheek teeth enamel have a number of peripheral folds that extend the full crown length at the buccal, lingual, and the two interdental contact surfaces, with additional folds also present on the occlusal surface. The upper cheek teeth have two deep enamel invaginations termed the mesial (rostral) and caudal (distal) infundibula, which can vary from vase to funnel shape, depending on their vertical depths into the teeth. These infundibula extend for almost the full crown length and are usually incompletely filled with cement (St. Clair 1975; Baker 1985a).

The combination of wear resistant enamel with the 2 adjacent softer dental tissues (cement and dentine) gives rise a number of ridges (styles) and depressions (grooves) on the occlusal surface (fig 4), that are additional to the enamel foldings of the occlusal surface. These ridges and depressions on the occlusal surface of the upper cheek teeth correspond to ridges and grooves on the lingual (medial or palatal) aspects of the teeth (Baker 1985a). Each row of (6) cheek teeth contains of a total of

10 ridges, two on the occlusal surface of each tooth, except the first and last which contain only one ridge. These ridges and grooves forms an intricate pattern resembling the gothic letter "B", the upright stroke of it being on the lingual aspect of the upper cheek teeth surfaces (Hofmeyr 1960; Baker 1985a). The ridges and grooves of the upper and lower cheek teeth interdigitate when the mouth is shut.

In normal horses the distance between the maxillary rows is approximately 30% wider than that between the mandibular rows, which is termed anisognathia (Mueller 1991). Additionally, the maxillary cheek teeth are wider than their lower counterparts. Consequently, when the mouth is closed, approximately one-third of the occlusal surface of the upper cheek teeth are in contact with about half of the lower cheek teeth's occlusal surface.

There are also differences between the maxillary and mandibular teeth in shape, number of pulp cavities and corresponding dental stars. The maxillary cheek teeth are squarer and have 5 pulp cavities in contrast to their lower counterparts which are more rectangular and contain 2 main pulp cavities with 5-6 subdivisions (S. Kilic, personal observation).

2.3.7. Blood supply of teeth

In brachyodont teeth, the number, size and distribution of pulpal blood vessels varies from the coronal to the apical regions of the pulp and changes throughout the life of the tooth (Jones 1990). When the blood vessels first enter the pulp through the apical foramen they are usually accompanied by sensory and sympathetic nerves. However, some blood vessels, unaccompanied by nerves enter through small accessory foramina (Hayward 1981; Jones 1990). Soon after the arteries enter the pulp their walls become thinner and they form arterioles. Hayward

(1981) stated that arterioles proceed coronally along the periphery of the pulp. In contrast Torneck (1994) noted that they proceed in the middle of the pulp. At the pulp periphery (i.e. subodontoblastic area) these arteriole branches form an extensive capillary network, particularly in the coronal region of the pulp and some capillaries may loop through the odontoblastic layer (Jones 1990).

Blood capillaries drain into an extensive venous network which have a more tortuous course than arterioles and exits via the apical foramen (Jones 1990). Although the external diameters of all pulpal arterioles and venules are similar the internal diameter of venules is larger as their walls are thinner (Jones 1990: Torneck 1994). Anatomoses may occur between pulpal arterioles and venules (Hayward 1981; Torneck 1994).

2.3.8. Lymphatic supply of teeth

Due to difficulties in microscopically distinguishing them from blood vessels it remains unclear if lymph vessels are actually present in pulp (Jones 1990). However, Hayward (1981) believes that pulp tissues, like all other connective tissues contains lymph vessels that in humans, drain into the submandibular and deep cervical lymph nodes. Torneck (1994) presented some distinct features that identify lymph vessels from blood vessels, namely that lymphatics are small, blind walled vessels in the coronal aspect of the pulp and their walls have discontinuities, that unlike the fenestration of blood capillaries are not externally surrounded by a basal membrane.

2.3.9. Nerve supply of teeth

Because of its great importance in human dentistry, the innervation of teeth has been well studied in many brachyodont species. Nerves fibres enter the pulp via

the apical foramen either separately, or accompanied by blood vessels within neurovascular bundles. These nerves include both sensory and sympathetic fibres. The sensory nerves originate from the trigeminal (5th cranial) nerve and are composed of A-type myelinated and C-type unmyelinated axons. The sympathetic fibres are derived from the cervical ganglion and are formed only by unmyelinated or C-type axons (Jones 1990; Torneck 1994).

The unmyelinated fibres supply the smooth muscles of blood vessels and so regulate blood flow in the pulp (Hayward 1981; Jones 1990). Additionally they are believed to control the differentiation and function of odontoblasts, including their circadian rhythm (Jones 1990). The sensory nerves give off few branches until they reach the coronal region of the pulp. They then ramify extensively in the subodontoblastic area forming the plexus of Raschkow that can be demonstrated in silver stained sections on the LM and also by immunochemical techniques (Ten Cate 1994).

The type and duration of pain in dentine are believed to be different from those of the pulp. Dentine responds to various stimuli including excessive heat and cold and to dental procedures such as drilling, with a sharp pain which stops when these stimuli cease. In contrast, stimulation of the pulpal nerves produces dull pain, which continues for some time after the stimulus is removed (Jones 1990).

2.4. DENTAL DISORDERS

2.4.1. Abnormalities of number

Abnormalities of teeth number are commonly divided into three groups, termed adontia, oligodontia and polyodontia (Barker & Van Dreumel 1985). Congenital adontia has been rarely recorded in the horse (Miles & Grigson 1990), but

is a hereditary problem in calves where it is associated with skin defects (O'Connor 1950; Barker et al 1993).

Oligodontia has been occasionally reported in domestic animals including horses. Cook (1965) reported two cases, one with bilateral absence of the 5th mandibular cheek teeth (M2) and one with unilateral absence of three permanent upper PM germs accompanied by a maxillary sinusitis. The later case could be termed pseudo-oligodontioa if it occurred as a result of loss or failed eruption of teeth (Barker & Van Dreumel 1985).

Although supernumerary teeth can appear anywhere in the equine dental arcade in horses, they most frequently occur in the incisors, particularly the upper incisors (O'Connor 1950; Baker 1985b). Colyer (1906) recorded polyodontia in just 3 out of 484 (0.62%) horse skulls he examined. The presence of a supernumerary 7th upper cheek teeth has been reported by Dixon (1992).

2.4.2. Delayed eruption

Persistence of the temporary teeth may prevent or delay eruption of the permanent teeth and incisors are most commonly involved in this disorder (O'Connor 1950).

2.4.3. Temporary dental retention

Temporary dental retention (impaction) is evident when cyst-like swellings are observed in the ventral mandible, adjacent to the apices of the 3rd and 4th PMs in 3-4 year old horses. These swellings are believed to result from the mechanical expansion of rapidly growing apical dental tissues that are retained in their bony sockets. Temporary dental retention is more common in early maturing breeds (e.g. Thoroughbreds and Standardbreds) than late maturing breeds (e.g. draught horses).

These mandibular swellings usually disappear at 4-5 years of age when the mandibulum becomes large enough to accommodate the teeth. Occasionally, mandibular swellings can result in dental fistulae near the apices of PM3s or 4 and occasionally M1 (Eisenmenger and Zetner 1985).

2.4.4. Abnormalities of positioning (dental displacements)

Abnormalities of positioning include overlapping, transverse or oblique positioning and rotation on the long axis. The third permanent lower cheek tooth (PM2) is most commonly displaced (Baker 1991). These abnormalities may be associated with abnormal shortening of the mandibulum or maxilla, or rarely can be secondary to maxillary, mandibular or incisive bone fracture and maxillary cysts (Dixon 1992). Early removal of the deciduous tooth remnants (caps) may also lead to displacement of permanent teeth (O'Connor 1950; Barker & Van Dreumel 1985).

2.4.5. Malocclusions

Abnormal shortening or lengthening of the mandible, are respectively termed brachygnathism (overshot jaw, overbite or parrot mouth) and prognathism (undershot jaw, underbite or sow mouth) and can cause abnormal tooth wear. The reverse conditions are maxillary brachygnathia or prognathism. Mandibular brachgynathia is characterised by protrusion of the upper incisors rostral to the lower incisors and mandibular prognathism results in the protrusion of the lower incisor rostral to the upper incisors. These abnormalities are believed to be hereditary defects in horses (Baker 1991; Mueller 1991). Baker (1979) found that only 3 of 446 horses had parrot mouth and he claimed that this incisor abnormality was accompanied by cheek teeth abnormality, i.e. overgrowth of the first cheek teeth. Many authors, including Mueller

(1991) and Wintzer & Jaksch (1986) reported brachygnathism to be more common than prognathism.

2.4.6. Dentigerous cysts

Dentigerous cysts, also termed heterotopic polydontia or temporal teratoma, are believed to originate from Hertwig's epithelial root sheath or its precursor, the enamel organ (Baker 1991; Mueller 1991). They are generally found in the temporal region of horses and usually drain onto the rostral aspect of the ear pinna (Mueller 1991). They become clinically evident when the dentigerous cyst swells or its wall dilates in the young adult horse and then discharge a sebaceous secretion via a duct to the rostral margin of the ear pinna (Baker 1985b). These cysts may contain epithelial structures, such as enamel epithelium, alveolar bony remnants and even hair (Eisenmenger & Zetner 1985; Mueller 1991).

2.4.7. Abnormal dental wear (Acquired dental abnormalities)

Abnormalities of wear are a very common clinical problem in horses and include sharp enamel points, shear mouth, wave mouth and step mouth. Baker (1979) and Uhlinger (1987) respectively reported that 18% and 19.3% of horses have various wear abnormalities. Additionally, abnormal dental wear was found to exist in 47% of horses over 20 year of age and was commonly associated with periodontal disease and/or dental caries (Baker 1979). The latter condition defined as dissolution of the mineral components and disintegration of the organic components of dental tissues as a result of the acidic action of microorganism and their products (Baker 1974).

2.4.8. Sharp enamel points

Sharp enamel points commonly develop along the cheek teeth at the buccal aspect of the upper cheek teeth and at the lingual aspect of the lower cheek teeth (Baker 1985b) They are usually present bilaterally and can result in buccal and lingual lacerations (Lane 1994). The discrepancy in width between the upper and lower teeth arcades (anisognathia) along with the limited lateral motion of the equine temporomandibular join may predisposes to this abnormality (Hofmeyr 1960; Mueller 1991).

2.4.9. Shear mouth

Shear mouth, also termed "sheer mouth" or "scissor mouth" is basically an advanced form of enamel overgrowths where the clinical crowns become triangular in shape, are ineffective at grinding and cause oral lacerations and pain. The lingual aspect of the occlusal surface in the upper, and the labial aspect of the lower cheek teeth arcades can even be worn to gingival level (Eisenmenger and Zetner 1985; Baker 1991; Wintzer & Jaksch 1986).

2.4.10. Wave mouth

Wave mouth is the presence of enhanced valleys and ridges across the cheek teeth occlusal surface in the saggital plane. It is due to abnormal masticatory movements, resulting in the development of an uneven occlusal surface between opposing upper and lower cheek teeth and can be predisposed to sharp enamel points, dental fractures or displacements (Baker 1991).

2.4.11. Step mouth

Step mouth is the presence of marked rectangular variations in the height of individual cheek teeth in an individual arcades and can be due to hypoplasia, fracture, surgical removal or shedding of the opposing teeth (Baker 1985b).

2.5. ENAMEL

Enamel is the hardest tissue in the body. In brachyodont teeth it covers coronal dentine, tapering in to an end at the cervical region but in hypsodont teeth it extends the whole crown length.

2.5.1. Physical characteristics

Enamel is easily differentiated from dentine and cement by its shiny, white appearance. However, enamel itself is transparent and its colour is due to the colour of the underlying dentine (Sognnaes et al 1966; Warshawsky 1983). Enamel that is heavily mineralised and also the thin enamel in the cervical tooth region is most transparent and transmits the colour of the underlying dentine best (Boyde 1990). Although enamel is the hardest tissue of the body, it can be easily damaged by trauma because of its extremely brittle nature. However, this risk is greatly reduced by the presence of underlying dentine which, due to its relatively elasticity, is capable of absorbing excessive forces applied to the overlying enamel (Osborn 1981).

2.5.2. Functions

The function of enamel is to provide a wear resistant surface against the attrition (enamel-enamel wear) and abrasion (enamel-food-enamel wear) of mastication. Enamel, unlike all other dental tissues has no inherent repair mechanism and thus cannot heal after traumatic injuries or infection (Boyde 1990).

2.5.3. Chemical characteristics

By weight, brachyodont enamel is composed of 2-5 % organic and 95-96 % mineral components, with this latter feature responsible for its great hardness (Sognnaes et al 1966; Osborn 1981; Fawcett 1987).

2.5.4. Organic phase

Although the organic components of enamel are not fully characterised, they include tyrosine rich amelogenin, enamelins and small amounts of glycoproteins, glycosaminoglycans, citrate and lipids (Sognnaes et al 1966; Osborn 1981; Warshawsky 1983). When enamel is fully decalcified, EM examination of its minuscule, unsupported organic matrix shows a network of fine organic fibrils, however, this delicate matrix is often totally destroyed by the decalcification process (Osborn 1981; Harvey & Dubielzig 1985). This organic matrix is most evident in decalcified sections of partially mineralised developing enamel (Ten Cate 1994).

2.5.5. Inorganic phase

The inorganic phase of enamel consists of impure hyroxyapatite crystals (Ca5(PO4)3OH). If magnesium, strontium, lead or fluoride are present during enamel formation they can also become incorporated into the hydroxyapatite enamel crystals (Osborn 1981; Warshawsky 1983; Fortelius 1985). Apatite crystals are the basic structural units of all calcified tissues, including bone, dentine, cement and enamel (Koenigswald and Clemens 1992). In human teeth, these crystals occupy approximately 88 % of the total enamel volume with the remainder composed of water and organic components (Osborn 1981).

2.5.6. Enamel crystals

The crystals of both prismatic and interprismatic enamel are best examined by the freeze-fracture technique that allows examination of ultrastructural detail without causing artefactual distortion such as are caused by decalcification, fixation or dehydration of enamel (Bai & Warshawsky 1985). Enamel crystals can also be examined by SEM in untreated samples and the organic structures of enamel can similarly be examined in decalcified sections. However, there is no satisfactory technique to simultaneously examine both the calcified and soft tissue components and this causes difficulty in fully understanding the structural relationship between these two components (Warshawsky 1989).

Some authors including Frank & Sognnaes (1960) and Johansen (1964) have described enamel crystals as calcified fibrils by assuming that the dark central lines seen in crystals are organic fibrils. However, Ronnholm (1962) claimed that these lines could be due the presence of higher molecular weight mineralised particles or to matrix proteins present in the crystals. Frazier (1968) found these lines present in only 20% of crystals, where they were symmetrically or diagonally located. EM examination of transverse sections of crystals with through-focus series revealed that these lines appeared at underfocus, disappeared at exact focus and became light in overfocus (Nylen et al 1963). Although the nature of these central dark lines remains unclear, it is generally believed at present that they are not due to the presence of calcified fibrils in the crystals.

Several authors including Travis & Glimcher (1964), Kallenbach (1986) described the organic matrix of enamel as a tubular sheath surrounding the crystals, from which they are separated by a thin light coloured zone on EM examination.

However, Leach (1979) believed that this organic coat merges with the crystal surface, with no such zone present between the organic sheath and crystal. Leach (1979) found the organic sheath to be 2.5-5 nm thick.

Bia and Warshawysky (1985) categorised enamel proteins into amelogenins and enamelins and proposed that the organic coat of crystals contained enamelins. It has been suggested that enamelins may initially act as template proteins to regulate the length, thickness and width of the enamel crystals during maturation. During crystal development, these proteins are displaced to and then remain at the crystal periphery.

Many authors including Ronnholm (1962), Frazier (1968), Leach (1979) and Warshawysky (1989) described recently formed enamel crystals as being thin, long and ribbon like on longitudinal section. Gordon (1976) claimed that these features make crystals highly fracture resistant, while Fortelius (1985) suggested that the combination of ribbon shaped crystal with intervening organic sheaths gives enamel some fibre-glass like characteristics.

Developing enamel crystals are initially separated from each other by large spaces that are oriented parallel to each other and to the main body of the enamel (Leach 1979). With progressive mineralisation, the crystals increase in thickness and thus the final size and shape of crystals depend on the size and shape of the available surrounding spaces. Enamel crystals contain protuberances and indentations along their lengths with adjacent crystals interdigiting with each other (Frazier 1968). Mature crystals are separated from each other by spaces of circa 1-2.5 nm wide (Leach 1979). Although several authors including Osborn (1981) and Warshawsky (1983) have noted human enamel crystals to be circa 0.2-1.2 μm long, Frazier (1968)

considered the measurement of crystal lengths to be unrealistic because of technical difficulties in examining the full crystal lengths in sections. Crystals can be deformed by the growth of adjacent crystals but these features can only be seen using the freeze fracture technique (Warshawysky 1989).

There is disagreement amongst workers regarding the transverse shapes of enamel crystals. Warshawysky (1989) has showed diagramatically how the plane of section can change the apparent relationship between the organic matrix and crystals and stated that one reason for the disagreements on the actual relationship between the crystals and the organic components is a misconception about the transverse shape of the crystals.

Mature human enamel crystals were described as being rectangular on cross section by Ronnholm (1962), but as having a flattened hexagonal shape by Frazier (1968). Bovine enamel crystals were defined as tablet-like boxes by both Ronnholm (1962) and Travis & Glimcher (1964), but their transverse sections were described as rectangular shaped by Warshawysky (1989). Rat enamel crystals mainly appear as hexagonal or occasionally as rectangular shaped, but neither represents the true transverse shape (Warshawysky 1989). He examined the transverse shapes of crystals before and after tilting enamel specimens on the microscope stage and found crystals that were initially rectangular became hexagonal in shape. However, crystals that were initially hexagonal shaped should conversely have become octagonal after tilting, but this inexplicably did not occur.

Developing enamel crystals are 25-30 nm wide on transverse section and 50-60 nm long in rats (Nylen et al 1963); 15 nm by 40 nm respectively in cattle (Travis and Glimcher 1964) and in humans 20 nm by 40 nm (Ronnholm 1962). Mature

human enamel crystals are between 10-90 nm wide and between 20-180 nm long (Frazier 1968). The hydroxyapatite crystals of enamel are larger than the inorganic crystals (also mainly hydroxyapatite) of the other 3 calcified tissues, namely dentine, cement and bone (Frank 1979).

Fossil examinations have shown primitive enamel to be aprismatic (nonprismatic) with its crystals extending from the amelodentinal junction to the occlusal surface in a straight and parallel course. A layer of aprismatic enamel has also been found surrounding the prisms of some more highly evolved lipotyphlans and carnivores (Koenigswald & Clemens 1992). The crystals of prismatic enamel, unlike those of aprismatic enamel, are oriented in differing directions to form separate prismatic and interprismatic enamels. The orientation of crystals defines the various types of prisms (Poole & Brooks 1961) and differences in crystal orientation of prismatic and interprismatic enamel determines the different structural types of enamel (Martin 1990). The evolutionary and ontogenetic relationships between aprismatic and prismatic enamel are unknown (Koenigswald and Clemens 1992).

2.5.7. Enamel prisms

A prism is described as a unit or bundle of crystals partially or totally bound by a prism sheath (Koenigswald & Clemens 1992). Osborn (1981) and Boyde (1990) stated that enamel crystals are oriented parallel to the long axes of prisms, but in rat enamel Jodaikin et al (1984) and Washawsky (1989) found them diverging by up to 25° from the long axes of prisms. Although prisms are just bundle of crystals that have complete or incomplete boundaries, their shape and orientation are important features in characterising the different types of enamel.

Under light microscopy, human enamel prisms are perpendicularly oriented from the amelodentinal junction towards the occlusal surface of the teeth (Osborn 1981). They are tapered, with a diameter of circa 3 μm at the amelodentinal junction and circa 6 μm at the occlusal surface. In humans, the number of enamel prisms has been estimated to be approximately 5 millions in incisors and 12 millions in molar teeth (Sognnaes et al 1966).

Enamel prisms are usually enclosed by interprismatic enamel which has the same chemical composition, but different crystal orientation. Interprismatic enamel has a slightly higher refractive index than prismatic enamel and the ratio of these two tissues can vary between species and even between different regions of a tooth. In human teeth, more interprismatic enamel is present adjacent to the amelodentinal junction than at the occlusal surface. The enamel of other species including those of the pig and dog contain much interprismatic enamel (Sognnaes et al 1966). Prismatic and interprismatic enamel crystals are oriented at angles of between 45-90° to each other, depending on the enamel type (Jodaikin et al 1984; Washawsky 1989; Koenigswald and Clemens 1992).

Prism sheaths demarcate the boundaries of enamel prisms and occupy the spaces between differently oriented crystals of adjacent prisms. They are circa 1 μm wide and stain darker than the enamel prisms (Sognnaes et al 1966; Osborn 1981). They have a lower refractive index (1.3) than prismatic or interprismatic enamel (1.6) due to their lower mineral content (Osborn 1981).

Enamel prisms may form different spatial relationships (packing patterns) to each other (i) within various levels of a tooth, (ii) between teeth of the same species and (iii) between the teeth of various species (Boyde 1964; Grine et al 1987). The

first comprehensive SEM study on the transverse shapes and packing patterns of mammalian enamel prisms was undertaken in developing enamel by Boyde (1964). Boyde described three enamel types that were termed mammalian types (patterns) 1, 2 and 3, according to the transverse shape of prisms and the prism packing patterns. Boyde's type 1 enamel has complete prism boundaries, but her types 2 and 3 enamel have incomplete C (arcade) shaped prism boundaries (fig 5) when examined on oblique sections (Fortelius 1985). The incomplete side of the C shaped prisms correspond to the side of hexagonal shaped prism territory in Boyde's type 2 enamel, but to a corner of a hexagon in Boyde's type 3 enamel (fig 5). Boyde's type 2 prisms are oriented in columnar forms and are divided by variable amount of interprismatic enamel (fig 5), while Boyde's type 3 prisms are aligned in rows with no interprismatic enamel present between them (fig 5).

There is no absolute distinction between these three patterns and in particular between Boyde's types 2 and 3 and it is always possible to find intermediate forms, even in areas that contain mainly one enamel type (Fortelius 1985). On oblique sections, type 3 enamel prisms can also have "keyhole" shaped prisms which are particularly prominent in primate enamel (Martin et al 1988; Koenigswald and Clemens 1992; Kozawa 1992). Boyde's type 1 enamel is most commonly seen in primitive mammals, whereas Boyde's types 2 and 3 enamel predominate in more highly evolved mammals. This indicates that enamel structure not only defines different enamel types but also helps to define the degree of evolution of some species (Fortelius 1985).

Variations in prism shapes and prism packing patterns are due to factors including, the movement of ameloblasts across their secretions, the size of secretory

territories of ameloblasts, their rate of secretion (Fortelius 1985) and the shapes of their Tomes' processes (Koenigswald and Clemens 1992). Examination of developing teeth in various species has shown that the number of ameloblasts contributing to the formation of one enamel prism varies with the type of enamel. For example, in Boyde's type 1 enamel, individual prisms are surrounded by interprismatic enamel and therefore each ameloblast produces just one single prism. In Boyde's type 2 enamel, each prism is formed from the secretion of two ameloblasts, although each ameloblast contributes to two prism. In Boyde's type 3 enamel, four ameloblasts are responsible for the deposition of one prisms. Conversely, each ameloblast will contribute the formation of four prisms. Therefore, in all of Boyde's enamel types, the number of ameloblasts is always equivalent to that of prisms (Fortelius 1985). The rather complex mechanisms of enamel prism formation have been described diagramatically by many authors including Osborn (1981) and Boyde (1990).

Measurements of the transverse area of prism territories in developing enamel of several species including the horse showed that Boyde's type 1 enamel prisms (1.2-1.6 μm^2) were larger than Boyde's type 2 (0.7-1.2 μm^2) but were smaller than Boyde's type 3 (1.6-2 μm^2) enamel prisms (Boyde 1969). In primates, these variations may be due to relatively simple mechanical constraints such as overcrowding of ameloblasts in a fixed territory (Boyde 1964) or to difference in the secretion rate of enamel, e.g. type 2 is secreted slower (less than 2 μm/day) than type 3 (5-7 μm/day) (Fortelius 1985).

The presence of 2-4 nm wide pores called "molecular sieves" between enamel crystal has been noted by Poole et al (1961) and Warshawsky (1983). These pores

occupy approximately 0.1 % of the total enamel volume, although this can vary with the degree of maturation of enamel (Darling et al 1961), with immature enamel containing more pores than mature enamel. If ground enamel sections are immersed into a medium whose molecules are larger than these pores (e.g. quinoline and ethanol) and are then examined by transmission LM, these porous area appear dark due to air trapped within these pores. These dark areas disappear if these sections are immersed in a medium whose molecules are small enough to penetrate the pores and replace the trapped air (Darling et al 1961; Gustafson & Gustafson 1961).

2.5.7.1. Prism decussation

In evolved mammals, enamel prisms rarely follow straight courses, usually bending to different degrees throughout the enamel thickness. These deviations are usually more marked in the cuspal, than in the cervical regions of brachyodont teeth. During their courses, the directions of individual rows or bundles of prisms may vary by up to 90° from the direction of adjacent rows or bundles. SEM and x-ray diffraction studies have shown that in rat incisor enamel, the prism rows cross each other at angles of 60-80° (Jodaikin et al 1984). This variation in prism orientation is termed "prism decussation" and is due to divergence in the course of groups of adjacent ameloblasts during enamel formation (Boyde 1990). Prism decussation is regarded as a functional adaptation, i.e. to prevent cracking in parallel rows of prisms and thus to strengthen the enamel structure against masticatory forces (Osborn 1981; Boyde 1990; Rensberger 1992). The transverse shape of prisms and the pattern of prism decussation are believed to be interrelated and this relationship is most evident in the highly developed decussation pattern of rodent enamels (Boyde 1969).

Because of the complex orientation of their gross enamel folds, the analysis of stress distribution throughout hypsodont teeth is not as easy as is the case in brachyodont teeth. Stress distribution has been studied in hypsodont teeth of rhinocerotoids using a "finite element model" stresses (Pfretzschner 1992). Whilst this model recognises the stress distribution in transverse section of the tooth, it neglects circumferential stresses (Pfretzschner 1992).

2.5.7.2. Hunter-Schreger (H-S) bands

When human enamel is examined under low magnification using a polarised LM, it is found to contain alternating light and dark bands called Hunter-Schreger (H-S) bands which are also respectively known as diazone and parazone. Diazone (light areas) refers to bands whose prisms are sectioned transversely and parazone to bands with vertically (longitudinally) sectioned prisms (Boyde 1990). These bands extend from the amelodentinal junction for approximately two-thirds of the enamel thickness in human teeth (Osborn 1981). H-S bands are optical phenomenon caused by the presence of prism decussation and are due to different degrees of light absorption by prisms running in different directions (Boyde 1990; Eisenmann 1994). Dark zones appears when increased amounts of light are absorbed by prisms, which occurs when the incidence of light is parallel to the long axes of prisms. On the other hand, when the light incidence is vertical to the long axes of prisms, much light is reflected back and the area appears bright (Boyde 1990; Rensberger 1992).

Kozawa (1992) examined fractured M3 of the modern horse (*E. caballus*) and four of its ancestors and found H-S bands to be oriented almost horizontally to the occlusal surface in the earliest perissodactyl, i.e. *Hyracotherium*. H-S bands became marked in the outer enamel layer of *Mesohippus*, which was accompanied by a

change of prism orientation from alternating into a regular straight pattern, reduction of prism sizes and increase in prism numbers (Kozawa 1992). The patterns of enamel ultrastructure that are currently seen in hypsodont enamel actually evolved in *Mesohippus* which had brachyodont teeth. Therefore, it appears that evolutionary changes first appeared in the microstructure of teeth and was later followed by gross alterations from brachyodonty to hypsodonty (Kozawa 1992).

The horizontal H-S bands have evolved from horizontal to vertical in some developed Perissodactyls, particularly rhinoceroses and tapiroids, but this orientation is rarely found in horses (Rensberger and Koenigswald 1980). The evolution of hypsodonty has increased the surface area of enamel which results in an increased numbers of prisms. The marked increase in prism number and the consequent reduction of their sizes together with the development of vertical orientation of H-S bands may have increased the strength of hypsodont enamel (Kozawa 1992).

2.5.8. Incremental (growth) lines

Enamel, like dentine, contains a series of incremental lines which may be caused by subtle changes in (i) enamel composition, due to possible effects of systemic disturbances on ameloblasts activity, (ii) the thickness of prisms due to rhythmic ameloblastic activity and (iii) the periodic bending of prisms (Sognnaes et al 1966; Boyde 1990). The life history and development of individual teeth can be investigated by examination of these lines (Chomette et al 1988; Boyde 1990). The pattern and density of incremental lines give important clues to the strength of enamel tissue as they show where enamel is least resistant (Boyde 1990).

2.5.9. Cross striations

Human enamel prisms contain alternating varicosites and constrictions at approximately 4 μm intervals throughout their lengths. These varicosites and constrictions represents daily (circadian) variation of ameloblastic activities and are microscopically characterised by incremental growth lines that are called "cross striations" (Boyde 1990; Eisenmann 1994). Varicosites occur where ameloblasts are most active and secrete more matrix into enamel pits, whereas these conditions are reversed in constrictions. Variation in the size and molecular constituents of hydroxyapatite crystals, particularly its carbonate ratio can occur between successive incremental lines (Osborn 1981; Boyde 1990).

2.5.10. Striae of Retzius

Retzius striae, which are also termed brown striae of Retzius due to their brown colour on transmitted LM examination (Boyde 1990), can be identified in transverse sections of human teeth, where they resemble the growth rings of a tree trunk (fig 6) (Osborn 1981). In longitudinal sections of teeth, they surround dentine at the coronal region of teeth and are directed obliquely towards the cervical aspect (fig 6) (Sognnaes et al 1966). They extend to the enamel surface and form the bottom of perikyma (see below). These lines are more pronounced than cross striations and their thickness ranges from 2-100 μm. They are believed to represent weekly (circaseptimanian) variation in ameloblastic activities (Eisenmann 1994).

Microradiographic and polarised LM examinations suggest that Retzius striae may be due to variations in mineralisation of consecutive incremental layers or to differences in spacing between enamel crystals. Thick Retzius striae can also develop as a result of disturbances of enamel formation such as occur with systemic disease

(Osborn 1981). All Retzius striae are formed at the same time and therefore all teeth in an individual show a uniform pattern that can be used to forensically identify teeth (Boyde 1990).

2.5.11. Perikymata (pl, perikyma)

The unworn surface of brachyodont teeth show a number of furrows which are referred to as perikymata or imbrication lines. These are trough like depressions of various depth which have perikymata crests on their sides and a Retzius line at the bottom (fig 6) (Sognnaes et al 1966; Osborn 1981; Boyde 1990). They are more marked at the lateral and cervical aspects than on the occlusal surface of teeth. Perikyma can provide attachment for plaque and calculus formation in brachyodont teeth (Boyde 1990).

2.5.12. Enamel fissures

The occlusal surface of normal brachyodont teeth may have fissures (clefts) which can extend to varying depths into the enamel. They most commonly occur in multicusped teeth where enamel deposition starts at the tips of individual cusps and later should fuse. However, if this fusion is incomplete, an enamel fissure will extend from the occlusal surface to the amelodentinal junction and in humans, dentine in these regions is susceptible to caries (Osborn 1981; Boyde 1990).

2.5.13 Lamellae

Lamellae are thin, leaf-like organic sheaths that extend throughout the full thickness of enamel and may even extend into dentine. Three types of lamellae have been described in human teeth, namely (i) lamellae formed due to developmental faults that consist of poorly calcified prism segments, (ii) lamellae formed when enamel has split by compression during dental development with the resultant defect

being filled by reduced enamel epithelial cells and (iii) lamellae associated with traumatic cracks that are filled with oral debris and saliva (Osborn 1981). Lamellae can readily be confused with iatrogenic cracks that occur during cutting or grinding enamel specimens. In such circumstances, lamellae can be definitively identified by decalcification of the suspect sections, during which lamellae remain intact but artefactual cracks disappear (Osborn 1981).

2.5.14 Enamel tufts

Although their structure and origins are not fully understood, enamel tufts are believed to be hyperproteinized areas that fail to mineralise during enamel maturation (Boyde 1976; Thylstrup 1979). In mature brachyodont teeth they appear as thin ribbon-like structures that extend from the amelodentinal junction to up to one third of the depth of coronal enamel (Osborn 1981). Tufts commonly develop at the inner enamel layer of human teeth where enamel prisms strongly decussate. It is suggested that an abrupt change in the direction of prisms in this region may disrupt the mineralisation path of enamel resulting in less mineralised areas (Eisenmann 1994). The presence of tufts in strongly decussated areas of human enamel suggests that they may serve to prevent crack propagation in these regions (Boyde 1990).

2.5.15. Spindles.

Enamel spindles may be fossilised odontoblast processes that crossed the amelodentinel junction before the beginning of amelogenesis. They could also represent the spaces of ameloblasts that died in the early stage of enamel deposition and then became encapsulated by the matrix of surrounding ameloblasts (Boyde 1990). They are commonly seen at the tip of the cusps, where dentogenesis begins, extending from the amelodentinal junction to circa 30-40 μm into the enamel

(Osborn 1981). On ground enamel sections, spindles often disintegrate and are replaced with air and therefore, appear as dark areas on transmission LM (Sognnaes et al 1966).

2.6. DENTINE

Dentine is a calcified dental tissue produced by cells originating from the ectomesenchyme of the dental papilla. Mature dentine contains numerous, fine cytoplasmic extensions and therefore is considered a living tissue. Dentine and pulp are commonly termed the pulpodentinal complex because of their intimate embryological and functional relationships (Torneck 1994).

Predentine, the precursor of dentine lines the pulpal interface of dentine and is an unmineralised dentinal matrix containing abundant collagens, glycoproteins, proteoglycans, similar to osteoid in developing bone (Torneck 1994). The presence of larger collagenous fibres (diameter, 0.6-0.7 μm) in intertubular dentine as compared to predentine (0.4-0.5 μm) indicates that these protocollagen molecules continue to grow for a period after the initial fibre formation (Johansen & Parks 1962). Predentine ranges in thickness from 10 to 47 μm, being thicker where active dentinogenesis occurs (Torneck 1994) and becomes completely calcified within 24 hours of its secretion (Baker 1979; Baker 1985a). Although Jones (1990) claimed that there is no evidence that odontoclasts can resorb mineralised dentine, Torneck (1994) suggested that dentine is vulnerable to resorption by odontoclasts when predentine is absent.

2.6.1. Functions

Dentine is the main constituent of teeth and underlies and supports the coronal enamel in brachyodont teeth. In hypsodont teeth, it additionally directly

contributes to the masticatory surface after the (temporary) occlusal aspects of cement and enamel are worn away (Shellis 1981; Jones 1990).

2.6.2. Physical characteristics

Dentine has yellowish white colour and is semitranslucent in vivo (Fawcett 1987; Mueller 1991). It is intermediate in hardness between enamel and cement and due to its relatively high collagen and proteglycans content, it has great compressive and tensile strengths. By absorbing masticatory and other external forces these characteristics of dentine compensate for the hard and incompressible features of the adjacent enamel and as previously noted, this helps prevent the brittle enamel from cracking (Shellis 1981). The physical and chemical characteristics of dentine resemble those of bone, except that dentine contains only odontoblasts processes with the odontoblast cell bodies being found at the pulpodentinal junction (Scott & Nylen 1966; Shellis 1981).

2.6.3. Chemical characteristics

Dentine is composed of 72 % mineral, 18 % organic matter and 10 % water (Shellis 1981). The mineral constituents of dentine include calcium and phosphate at about 1:55 ratio, sodium chloride and a ranges of trace elements (Shellis 1981). These minerals are found mainly in imperfect hydroxyapatite crystals which are similar to those of the other calcified dental tissues and bone (Scott & Nylen 1966). Radiographic diffraction and TEM (transmission electron microscopy) studies have shown the apatite crystals of dentine to be between 20-100 nm long and 2-2.5 nm thick, which are smaller than those of enamel (Shellis 1981). A TEM study, using an ion-beam etching technique, which avoids mechanically stressing crystals as occurs

with diamond knife sectioning, indicated that dentinal apatite crystals could be up to 65 nm long (Boyde 1974).

The shape of dentinal crystals is unclear, being described as needle-like by Takuma (1960), cigar-like by Pouter (1960) and as plate-like by Johanson and Parks (1962). Boyde (1974) noted that they appear needle-like if mineralisation occurs along microfibrils, but plate-like if these microfibrils join together.

Collagenous fibres constitute approximately 92 % of the organic components of dentine (Fawcett 1987), but these collagen fibres are usually masked by the dense aggregation of hydroxyapatite crystals (Warshawsky 1983). The ground substance of dentine has a slightly lower organic content than bone, containing phosphoproteins, proteoglycans, glycoproteins proteins, some plasma proteins, glycosaminoglycans, lipids and organic acids (Scott & Nylen 1966; Shellis 1981; Jones 1990; Torneck 1994).

2.6.4. Incremental lines

Dentine deposition continues in an incremental pattern throughout the life of a tooth. The presence of incremental lines indicates that histological changes, caused by factors including variations in mineralisation, the orientation of its collagenous fibres and in the shape and orientation of dentinal tubules occur between different dentinal regions (Shellis 1981; Fawcett 1987).

Lines of von Ebner represent diurnal variation in the deposition and arrangement of collagenous fibrils in successive layers of dentine (Schroeder & Frank 1985; Shellis 1981). In humans, von Ebner lines appear as curvilinear appositional growth lines (Fawcett 1987) with intervals between successive lines recorded as 5 μm by Shellis (1981) and 15 μm by Torneck (1994). Another type of

incremental lines found in circumpulpal dentine are the Contour lines of Owen which are thought to be formed by the coincidental alignment of the secondary curvatures of dentinal tubules (Shellis 1981; Torneck 1994). However, incremental lines caused by mineralisation deficiencies are also termed as Contour lines of Owen by Ten Cate (1994).

Dentine can be divided into two distinct types, called primary and secondary dentine. Secondary dentine can also be subdivided into regular (physiologic) and irregular (pathologic or reparative) dentine (Scott & Nylen 1966; Shellis 1981; Jones 1990). However, Stanley et al (1966) and Torneck (1994) have used the term tertiary dentine in place of irregular secondary dentine.

2.6.5. Primary dentine

Human primary dentine has a number of structurally different regions termed mantle dentine, the hyaline layer and circumpulpal dentine.

In human teeth, mantle dentine is 10-15 μm in thickness and surrounds the coronal aspect of circumpulpal dentine (Shellis 1981). The matrix of mantle dentine is laid down by preodontoblast cells of the dental papilla. The mantle dentine contains large (0.1-0.2 μm) diameter collagen fibrils oriented almost parallel to its dentinal tubules (Torneck 1994). It contains the ends of dentinal tubules and is slightly less mineralised than the remaining dentine. This latter feature is attributed to the absence of phosphophoryn within its ground substances, because this substance influences the degree of mineralisation of dentinal tissue (Takagi & Sasaki 1986).

The hyaline layer is located between cement and the granular layer of Tomes (see 2.4.2.7.3.). The collagenous fibres of the hyaline layer are oriented obliquely to the dentinal tubules (Shellis 1981). In brachyodont teeth, the hyaline layer extends

from the cervical region to the tooth apex, binding dentine to cement. The hyaline layer has always been considered as a part of dentine, because it was believed to be an odontoblast cell product (Shellis 1981; Jones 1990), however, recent studies have shown that it is actually formed by Hertwig's epithelial root sheath cells (Torneck 1994).

In brachyodont teeth, a hypomineralised zone is present between the hyaline layer and circumpulpal dentine and extends from the cervical region towards the tooth apex. This zone is called the granular layer of Tomes because it has a dark granular appearance on ground sections examined by transmission LM (Shellis 1981). The dark coloration occurs when light is refracted by the microscope lens. If the microscopic illumination is changed from transmission to incident, this layer becomes light in colour, because the light is refracted by air within the tubules. This granular layer is not observed in H&E-stained sections nor on EM examination (Torneck 1994). This zone may also result from light being scattered by air trapped in dilated terminal branches of the dentinal tubules (Osborn 1981).

Circumpulpal dentine forms the bulk of dentine and contains several distinct structures including dentinal tubules, peritubular dentine, intertubular dentine interglobular dentine and odontoblast processes.

2.6.6. Dentinal tubules

2.6.6.1. Orientation of dentinal tubules

Dentinal tubules radiate out from the pulp toward the amelodentinal junction, following an S-shaped course, particularly in the coronal dentine of human teeth (Torneck 1994). This S-shaped curvature is called the primary curvature. It is less pronounced in human root dentine, especially in the cervical area and also in the

crown, where the dentinal tubules run in an almost straight course beneath the incisal edges and cusps (Leeson & Leeson 1970; Shellis 1981). Small regular undulations are also superimposed on the primary tubule curvatures and are termed secondary curvatures (Shellis 1981). Human dentinal tubules contain lateral branches at intervals of 1 to 2 μm along their length (Torneck 1994). In human dentine, some tubules divide in two near the amelodentinal junction, with one tubule occasionally containing rounded bodies of 0.1-0.3 μm diameter. Similar round structures were also seen on the fractured surface of peritubular and intertubular dentine (Brannstrom and Garberoglio 1972).

2.6.6.2. Diameter and numerical density of dentinal tubules

There have been many studies on the numerical density (i.e. number of tubules in a unit area) and diameter of dentinal tubules in decalcified and undecalcified sections of human dentine. LM examination of decalcified human incisors found the numerical density of dentinal tubules to be 16.000, 60.000 and 70.000 per mm^2, respectively, near the amelodentinal junction, midway to the pulp and adjacent to the pulp (Ketterl 1961) and in undecalcified human premolars to respectively be between 13.458-22.244 , 33.819-43.177 and 40.257-61.587 (Fosse et al 1992). An SEM study of undecalcified human dentine reported values of 20.000 tubules/ mm^2, 29.500 and 45.000 at these 3 sites (Garberoglio & Brannstrom 1976).

All these studies show that the number of dentinal tubules increases from the amelodentinal junction to the pulp cavity and the ratio of dentinal tubule number adjacent to the amelodentinal junction: adjacent to the pulp dentine interface are 4.4 (Ketterl 1961), 2.3 (Garberoglio & Brannstrom 1976) and 2.8 (Fosse et all 1992). Recent studies in human teeth have also shown that tubular density is higher on the

lingual and buccal walls of the pulp than on the mesial and caudal walls (Schellenberg et al 1992). The recorded variations in numbers of tubules between different dentinal regions are believed to be due to alterations in the ratio of the peritubular: intertubular dentine, in the packing pattern of tubules and to crowding of odontoblasts toward the pulp surface (Ketterl 1961; Shellis 1981; Fosse et al 1992; Torneck 1994).

SEM examination of young human premolars showed tubular diameter to be 1.0 μm near the amelodentinal junction, 1.5 μm at a distance of 1 mm from the pulp and 1.8-2.0 μm near the pulp (Brannstrom and Garberoglio 1972). Garberoglio & Brannstrom (1976) found the diameter of dentinal tubules to be 0.9 μm near the amelodentinal junction, 1.5 μm midway to the pulp and 2.5 μm near to the pulp and Ketterl (1961) found values of 1, 4 and 5 μm, respectively. A TEM examination of ultrathin undecalcified dentine sections at these sites showed that tubule diameter was 1.77 μm near the amelodentinal junction and 1.8 μm μm near the pulp in young teeth and corresponding values were 1.21 and 1.54 μm in mature teeth (Fromme and Riedel 1970). The recorded differences between these studies could in part be due to differences in the processing techniques used, e.g. decalcification dissolves peritubular dentine which results in larger tubule lumina (Garberoglio & Brannstrom 1976). This increase in diameter of dentinal tubules from the amelodentinal junction to the pulp is believed to result from the decrease of peritubular dentine in this direction (Blake 1958; Takuma 1960; Fosse et al 1992).

Hoppe & Stuben (1965) examined decalcified coronal dentine sections of human permanent premolars and found a dentinal tubular volume: total dentinal volume proportion of 27.7% near the pulp and 19.1% near the amelodentinal

junction. However, a further study found values of 28% and 4% respectively for these sites and additionally found that tubules occupied circa 10 % of total coronal dentine volume (Garberoglio and Brannstrom 1976). These two studies differ greatly in tubular volume ratios near the amelodentinal junction. This differences may be due to the use of decalcified sections in the former study, where peritubular dentine was largely dissolved and thus the tubule lumina iatrogenically became larger than those of the latter study that used undecalcified sections. As peritubular dentine is not found adjacent to the pulp, the diameter of tubules in this region does not increase following decalcification. Therefore, the ratio of tubule volume: total dentinal volume near the pulp will be similar in both decalcified and undecalcified sections (Garberoglio & Brannstrom 1976).

Hildebolt et al (1986) measured the numerical density of dentinal tubules per unit area and the distances between adjacent dentinal tubules in humans, baboons, and dogs and found specific values for each species and have suggested that such measurements may have taxonomic value (Fosse et al 1992). However, another study of numerical densities of dentinal tubules in monkeys, rats, cats, dogs and humans found no such interspecies differences (Forsell-Ahlberg et al 1975).

2.6.6.3. Presence of a dentinal tubule membranous lining

Many workers have examined the structure and contents of dentinal tubules however they have obtained different findings. The first disagreement concerns the presence of a membranous structure lining the inner aspects of the dentinal tubules. An EM study of decalcified dentine has shown a membranous structure to surround the odontoblast processes throughout the dentinal tubular lengths (Arwill & Bloom 1954). However, Scott & Nylen (1966) who also used an EM to study enamel and

dentine, claimed that no such a membrane exists on the inner walls of dentinal tubules. Johansen and Parks (1962) detected a membranous structure in dentinal tubules of mineralised dentine, but not predentine. In total contrast, Brannstrom and Garberoglio (1972) found that a membrane lined the walls of dentinal tubules only in predentine and the inner 0.2 mm of the mineralised dentine. The remainder, i.e. the middle and outer aspects of the dentinal tubules that did not contain such a membrane were coated with a smooth mineralised layer of 0.1-0.2 μm in thickness. A histochemical study of decalcified dentine showed a heavily stained layer between the odontoblast processes and the tubule walls (Symons 1962) and Symons suggested that this layer could be either a thin limiting boundary of the peritubular dentine or the cytoplasmic membrane of odontoblast processes.

The membranous structure claimed to exist on the walls of dentinal tubules by previous workers has been termed the lamina limitant by Torneck (1994). He observed that this structure was only detected in examinations of decalcified sections but never in cryofixed or untreated sections. Consequently, Torneck proposed that this membrane might be an artefactual structure produced by chemically denatured organic components such as proteoglycans, tenascin, serum proteins, ∝-2 HS, or transferrin that are present in tubules. This dispute remains unresolved.

2.6.6.4. Collagenous fibres in dentinal tubules

Takuma (1960) found that collagenous fibres in dentinal tubules were oriented circumferentially or longitudinally and were embedded in the walls of these tubules. Brannstrom and Garberoglio (1972) agreed with this orientation of the collagenous fibres, but found the fibres located in the membranous structure rather than embedded in the walls of dentinal tubules. Torneck (1994) found that the

quantity of collagenous fibres increases toward the pulp and noted that their presence may limit the examination of odontoblast processes within the dentinal tubules.

2.6.6.5. Distribution of odontoblast processes

As odontoblasts lay down dentine they move towards the pulp cavity, but their basal ends gradually become narrow and remain in the tubules forming the cytoplasmic processes. These odontoblast processes can be seen crossing predentine and entering the dentinal tubules of the mineralised dentine. However, it remains unresolved whether the odontoblast processes extend as far as the amelodentinal junction or if they fill the entire lumina of the tubules. Some early studies including those of Syrrist & Gustafson (1951) and Arwill & Blood (1954) reported that these processes extended to the amelodentinal junction and totally filled the dentinal tubules. Syrrist & Gustafson also claimed that these odontoblast processes were so firmly attached to the walls of the dentinal tubules that no potential spaces remained in the tubules.

Frank (1959) recorded that odontoblast processes fully filled the lumina near the pulp dentine and were tubular-like in the middle and outer (adjacent to amelodentinal junction) dentine. However, Lester and Boyde (1968) challenged the traditional belief that odontoblast processes extended to the amelodentinal junction. In an SEM study of fractured human dentine they found that odontoblast processes extended no further than 0.7 mm from the pulpodentinal interface, Beyond this point, (i.e. in the middle and outer dentine) the lumina of tubules were empty. These findings have been supported by many further EM studies, including those of Tsatsas & Frank (1972) and Brannstrom & Garberoglio (1972). The latter authors found that most tubules contained odontoblast processes that usually extended 0.2 mm into

calcified dentine, a few tubules had processes extending up to 0.4 mm, but that at 0.7 mm from the pulp, all tubules appeared empty.

Odontoblast processes were also observed to be absent in the peripheral aspects of murine (Jessen 1967; Garant 1972) and feline (Holland 1975, 1976) dentine. The absence of odontoblast processes in the periphery of dentine could be due to the presence of contractile proteins such as tenascin (a glycoprotein associated with motility) in the odontoblast processes (Tucker et al 1991). When odontoblast processes are stimulated by various manipulative procedures such as clinical dental treatments or preparation of teeth specimens for microscopic examination, these proteins may cause retraction of the processes. Additionally, Holland (1976) ruled out this possibility, because he believed that the lateral branches of odontoblast processes embedded in the peritubular dentine would prevent such retraction. However, when dentine was cryofixed, which is believed to eliminate the effect of tenascin, odontoblast processes were observed near the amelodentinal junction (Kelly et al 1981). This finding is supported by later immunocytochemical studies (Sigal et al 1984; Sigal et al 1985).

A debate also exists concerning the presence of fluid in dentinal tubules. As previously noted, Syrrist & Gustafson (1951) believed that there were no spaces in dentinal tubules for such fluid. However, Brannstrom (1968) claimed that dentinal tubules contained a liquid of interstitial fluid characteristics. i.e. rich in sodium and low in potassium. Brannstrom and Garberoglio (1972) suggested that dentinal fluid accounted for 25 % of the total dentinal volume and as dentinal tubules occupy 21 % of the total dentine volume, most of this fluid should be present in the tubule lumina. Haljamae and Rockert (1970) collected fluid from exposed dentine in vivo and found

two types of fluid, one with an electrolyte composition similar to that of extracellular fluid. Despite these reports Torneck (1994) claims that there is still no conclusive evidence verifying the presence of extracellular fluid in the tubule lumens. He also indicated that as the collection of dentinal fluid in previous studies was achieved after dentinal exposure, this fluid could possibly be an inflammatory exudate resulting from damage to odontoblast cell bodies and processes (Torneck 1994).

Some tubules of mineralised dentine were found to contain nerve fibres by Frank (1959). However, Brannstrom and Garberoglio (1972) did not find nerve fibres extending beyond the junction of predentine and mineralised dentine. Dahl & Mjor (1973) claimed to have found nerve-like structures closely associated with odontoblast processes in the dentinal tubules of mineralised dentine. In human dentine, the number of tubules containing nerve fibres decreases from crown to apex and from the dentine pulp interface to the mineralised dentine. Nerve fibres have not been identified in the tubules of root dentine. In coronal dentine of human molars, the number of tubules with nerve axons was 14 % in predentine, 6 % at the mineralisation front, and 2 % in mineralised dentine. Nerve fibres, most of which are unmyelinated axons have not been identified in dentinal tubules beyond the point where the peritubular dentine first appears in dentinal tubules (Torneck 1994).

Torneck (1994) believed that nerve fibres may enter the dentinal tubules by chance. He explained that after nerve fibres reach the peripheral pulp, they encounter a barrier formed by the odontoblast layer and predentine and consequently most of them retract, forming the plexus of Raschkow in the subodontoblastic area. Meanwhile, some nerves may by chance approach the territories of tubules and enter between the tubular walls and odontoblast processes. Once they enter the tubules,

they can readily advance in the lumina until they reach a second obstruction formed by the peritubular dentine. At this point the lumina of the tubules become much narrower, thus preventing these fibres from advancing further (Torneck 1994).

6.6.7. Peritubular dentine

2.6.7.1. Nomenclature of peritubular dentine

The dentinal tubules are surrounded by ring-like structures that based on its LM, microradiographic and EM appearance have been described variously as (i) calcified canalicular sheaths (Shroft et al 1954), (ii) peritubular translucent zones (Miller 1954; Takuma 1958; Frank 1959), (iii) translucent areas (Bradford 1958), (iv) peritubular matrix (Takuma et al 1966) and (v) peritubular dentine (Fearnhead 1957; Bradford 1963; Tsatsas & Frank 1972; Schroeder & Frank 1985). The term peritubular dentine, first introduced by Fearnhead (1957) is currently most commonly used, although Torneck (1994) has recently claimed that the term peritubular dentine is anatomically incorrect and should be replaced by intratubular dentine.

2.6.7.2. Deposition of peritubular dentine

Peritubular dentine was initially thought to be formed by the odontoblast processes after tooth eruption, in response to noxious external stimuli such as caries (Blake 1958). This theory has been disregarded, because peritubular dentine has been found in healthy human teeth (Fosse et al 1992) and recently erupted (unworn) cervid (deer) teeth (Kierdorf & Kierdorf 1992). Torneck (1994) has proposed three theories on how peritubular dentine is formed. The first is that peritubular dentine may be a mineralised organic component which previously existed around the odontoblast processes. The second is that the odontoblast processes may secrete an organic matrix that is mineralised as a result of odontoblast activity. The third is that the

odontoblast processes may secrete an organic matrix that is mineralised as a result of passive redistribution of mineral ions from the intertubular dentine.

2.6.7.3. Organic and inorganic phases of peritubular dentine

The collagenous fibres of peritubular dentine are sparse and are oriented parallel to the dentinal tubules (Shellis 1981). Their diameters are 0.25-0.5 μm, which is smaller than the fibres of intertubular dentine (0.6-0.7 μm) and of predentine (0.45-0.5 μm) (Johansen and Parks 1962).

EM (Frank 1959; Takuma 1958; Johansen & Parks 1962), LM (Fosse et al 1992), x-ray microradiographic studies (Miller 1954; Bradford 1963) and electron probe microanalysis (Takuma et al 1966) confirm that peritubular dentine is more highly mineralised than intertubular dentine. Takuma et al (1966) examined an unspecified number of fractured equine molars utilising electron probe microanalysis and found that peritubular dentine contains almost twice as much mineral as intertubular dentine. The highly mineralised characteristic of peritubular dentine is believed to increase dentinal wear resistance (Bradford 1967). As equine dentine contains a high amounts of peritubular dentine, it is easier to study the ultrastructure of equine peritubular dentine than most other mammalian dentine (Takuma et al 1966).

Several authors including Frank & Voegel (1978) and Schroeder & Frank (1985) have examined individual crystals of human peritubular dentine using high resolution TEM and found that these crystals had a mean length of 36.00 ± 1.87 nm, width of 25.57 ± 1.37 nm and thickness of 9.79 ± 0.69 nm indicating that these are hexagonal on transverse sections. These authors also measured the periodic equidistant fringes of these crystals and found that these values correspond to those

of hydroxyapatite. These observations contradict earlier suggestions that peritubular dentine consists of closely packed spherical particles of circa 25 nm in diameter (Lester & Boyde 1968).

2.6.7.4. Amount of peritubular dentine

Examination of thin ground dental sections showed that peritubular dentine exists in many orders including, Primates, Carnivora, Perissodactyla and Artiodactayla but not in Insectivora and Rodentia (Bradford 1967). The relative volume of peritubular dentine differed even within species of the same mammalian order. Highest amounts of peritubular dentine were found in representatives of Artio- and Perissodactyla. This characteristic is believed to develop as an adaptive purpose to enhance dentinal wear resistance in teeth with limited growth (Bradford 1967).

An SEM study of teeth of fallow deer (*Dama dama*), roe deer (*Capreolus capreolus*) and wild boar (*Sus scrofa*) demonstrated significant variations in the volume and distribution of peritubular dentine between different dentinal regions. These variations were regarded as important characteristics in achieving and maintaining a functional occlusal surface (Kierdorf & Kierdorf 1992). In fallow and roe deer, the outer regions of the mid and cuspul coronal dentine contained high amounts of peritubular dentine in which dentinal tubules were non-centrally located, whilst this feature was confined to the more peripheral areas of cusp and flank (side) dentine in wild boars. Overall, the tubular asymmetry was less pronounced in wild boars than in cervids (Kierdorf & Kierdorf 1992). These variations may be related to the morphology of the occlusal surfaces and the particular mode of jaw movement which was reported to be transverse in cervids and ortal in pig (Fortelius 1985; Kierdorf & Kierdorf 1992).

Several authors have examined the amounts of peritubular dentine along the course of the dentinal tubules. Blake (1958) claimed that the volume of human peritubular dentine remained fairly constant from the amelodentinal junction to the pulp cavity. However, Takuma (1960) found that the volume of peritubular dentine increased from the amelodentinal junction to midway towards the pulp and then decreased. Brannstrom and Garberoglio (1972) found that peritubular dentine did not extend for the full length of dentinal tubules, but first appeared at about 0.2 mm from the pulp and reached 0.5 μm in thickness at 0.3 mm from the pulp.

Examination of thin undecalcified sections of human permanent teeth by stereoscopic microscope showed the diameter of peritubular dentine to be 60 nm near the amelodentinal junction and 80 nm near the pulp (Johanson and Parks 1962), but Fosse et al (1992) and Torneck (1994) found that the diameter of peritubular dentine decreased significantly between the amelodentinal junction and the pulp.

2.6.8. Dentinal sclerosis

Occlusion of the lumina of several tubules in a unit area is termed dentinal sclerosis. Sclerotic dentine has a glassy appearance on transmission LM examination of ground sections and is brighter in colour than normal dentine (Scott & Nylen 1966; Torneck 1994). Dentinal sclerosis was believed to develop for defensive purposes to prevent bacteria or their products from reaching the pulp cavity (Johanson & Parks 1962; Shellis 1981). However, the finding of dentinal sclerosis in unerupted human teeth which have been protected from all external influences casts doubt on this theory (Torneck 1994). Peritubular dentine becomes more calcified in older teeth, which also show progressive lumen obliteration, eventually leading to total dentinal sclerosis (Tsatsas & Frank 1972).

2.6.9. Organic and inorganic phases of intertubular dentine

The organic part of intertubular dentine is mainly composed of bundles of collagenous fibres that run in a criss-cross fashion, parallel to the mineralisation front of dentine and at right, or occasionally, at oblique angles to the dentinal tubules (Shellis 1981). The organic fibres of intertubular dentine in circumpulpal dentine are reported to be 0.6-0.7 μm in diameter by Johansen and Parks (1962) and 0.05-0.2 μm by Torneck (1994). Additionally, intertubular dentine contains more collagen fibrils than peritubular dentine (Johansen and Parks 1962; Torneck 1994). Two types of mineralisation have been found in intertubular dentine, namely linear pattern calcification and calcospherites (Shellis 1981; Jones 1990).

More than 56 % of dentine crystals are aligned parallel to the collagenous fibres of the dentinal matrix in a crystal-collagen complex, i.e. linear pattern mineralisation and this type of calcification may be initiated within the fibres themselves (Shellis 1981). In linear pattern mineralisation, where the crystal-collagen complex is intact, the calcified dentinal fibres shows alternating light and dark bands of circa 0.60-0.7 μm in length (Johanson and Parks 1962).

Calcospherites develop in focal areas, independently from the collagenous fibres and then unite in a interpenetrating system with the crystals aligning along the fibrils. The presence of calcospherite crystals are a specific feature that allows dentine to be readily differentiated from bone and cement, whose crystals are always aligned in a linear pattern (Shellis 1981). Two types of calcospherite patterns termed spherical and arcade (C shaped) have been identified in human dentine (Shellis 1981; Jones 1990).

Spherical pattern mineralisation is seen in mantle dentine, the hyaline layer, and also in the peripheral and inner two thirds of circumpulpal dentine (Shellis 1981). An SEM study of human intratubular dentine found these isodiametric spherical crystals to be approximately 2.5 nm in diameter (Lester & Boyde 1968). In the arcade pattern, the concavity of each arcade of calcospherites usually faces the tooth periphery, with their convexities facing the pulp. This pattern is found in the centre of circumpulpal dentine.

Mantle and peripheral circumpulpal dentine are noted to be less calcified than the other dentine types, which may be due to very rapid dentine formation at these sites (Shellis 1981) or to their lack of phosphophoryn (Takagi & Sasaki 1986). The mineralisation of dentine is complete when all of its calcospherites fuse together. If they fail to fuse, unmineralised or hypomineralised areas called interglobular dentine (spaces) remain in dentine (Shellis 1981). These lesions are common in human teeth where vitamin D deficiency or fluorosis existed during dentine formation and may be also due to very rapid formation of dentine (Shellis 1981). Interglobular dentine is most frequently observed in the circumpulpal dentine, just below the mantle dentine, where mineralisation is largely due to spherical calcospherites (Torneck 1994). As interglobular dentine is a defect of mineralisation, but not of matrix formation, dentinal tubules pass uninterruptedly through these areas (Tsatsas & Frank 1972; Torneck 1994). On transmission LM examination of ground sections, interglobular dentine appears dark due to the presence of air trapped within it (Scott & Nylen 1966). Calsospherites situated in interglobular dentine are readily visible on LM because of the difference in refractive index between them and the interglobular spaces.

2.6.10. Secondary dentine

Secondary dentine is regarded as a continuation of the primary dentine (Torneck 1994) and its deposition starts after tooth formation is complete, i.e. when a tooth reaches full occlusal contact (Kierdorf & Kierdorf 1992). However, some recent studies showed that secondary dentine occurs in unerupted human teeth that have not been exposed to any mechanical stress (Torneck 1994). The incremental line pattern and the size and shape of dentinal tubules in secondary dentine slightly differ from those of primary dentine. However, the major difference between primary and secondary dentine is that the courses of dentinal tubules change abruptly when they enter secondary dentine (Jones 1990). This change is marked histologically by the presence of an thick incremental line between primary and secondary dentine (Jones 1990). In addition, secondary dentine is not laid down as regularly on the pulp surface as is primary dentine (Scott & Nylen 1966). There is evidence that sclerosis develops more readily in secondary than primary dentine, thus reducing its permeability and thereby protecting the pulp (Torneck 1994).

2.6.11. Tertiary dentine

Tertiary dentine, also known as irregular secondary or reparative dentine is formed in response to noxious stimuli including excessive wear, caries, restorative dental procedures and fractures of dentine (Shellis 1981; Kierdorf & Kierdorf 1992). The appearance of and amount of tertiary dentine depends on the degree and duration of the stimuli. Severe stimuli may causes such extensive odontoblast degeneration that they cannot produce further dentine. In this situation, the parietal (peripheral) cells of pulp move towards the affected dentinal region and rapidly differentiate into odontoblast like cells that are capable of rapidly producing (almost 3.5 μm daily)

tertiary dentine to protect the underlying pulp. This type of tertiary dentine which is sometimes called osteodentine, contains sparse and irregularly sized dentinal tubules.

The low number of dentinal tubules present in tertiary dentine is attributed to rapid deposition of tertiary dentine, with most odontoblast-like cells becoming trapped in their own secretions. On the other hand, less severe stimuli causes slower deposition of tertiary dentine that has a relatively high number of regular dentinal tubules, but less cellular inclusions, as compared to the previous type. There is no continuity between the dentinal tubules of tertiary dentine and of the overlying primary or secondary dentine because tertiary dentine is formed by different cells. This feature minimises the permeability of tertiary dentine at the site of deposition and thus provides further protection to the underlying pulpal tissue (Torneck 1994).

The organised tubular orientation of primary and secondary dentine is believed to be due to the influence of the internal enamel epithelium on odontoblast at the beginning of dentinogenesis. As the internal enamel epithelium is absent where tertiary dentine is laid down, odontoblasts in such area cannot align regularly, which results in the irregular tubular orientation of tertiary dentine (Osborn 1981). In addition to the number, size and organisation of dentinal tubules, tertiary dentine also differs from both primary and secondary dentine by containing type I and II collagens which further confirms that tertiary dentine is produced by different cells.

2.7. CEMENT

2.7.1. Definition

Cement (cementum) is a specialised calcified tissue of mesodermal origin with mechanical characteristics and a histological appearance resembling bone, particularly alveolar bone (Selvig 1965; Osborn 1966).

2.7.2. Functions

Cement provides an anchorage for fibres of the periodontal ligament, protects the underlying dentine at the tooth apex, and particularly in hypsodont teeth (Dixon and Copeland 1993) it contributes to the size and strength of the teeth to compensate for crown wear. The resorption and deposition of cement can contribute to the apposition of the teeth and maintain occlusal contact of the dental arcades during their functional life. As a component of the periodontium, cement is essential for normal eruption, support and maintenance of teeth. In hypsodont teeth, coronal cement additionally directly contributes to the occlusal surface (Osborn 1966; Jones 1981). Alteration in any periodontal tissue can stimulate cementoblasts to produce cement and this continued activity of surface cementoblasts makes cement the most flexible of the dental tissues (Osborn 1966).

Cement deposition continues throughout a tooth's life, around both roots and reserve crowns of hypsodont teeth and around the roots of brachyodont teeth. However, the deposition of additional cemental layers does not enhance the strength of tooth attachment, since the embedded periodontal ligament fibrils (Sharpey's fibres) mineralise and become inflexible with increased cemental deposition (Selvig 1965; Osborn 1966; Jones 1981). On the other hand, continuing cemental deposition, along with continuing tooth eruption results in rearrangement of the sequential periodontal ligament fibrils, and it has been proposed that the rearrangement of periodontal ligaments is the actual mechanism of tooth eruption (Osborn 1981). This rearrangement is believed to occur due to the gradual rebuilding of collagenous fibrils (Selvig 1965; Zander 1966).

2.7.3. Physical and chemical characteristics

Cement can be easily distinguished from enamel or dentine by its pale cream colour. It is composed of 65% mineral and 35% organic material. Collagens make up 70 % of the organic constituents of human cement (Jones 1981). Like other calcified tissues, most of the mineral component of cement is composed of impure hydroxyapatite crystals (Selvig 1965). In developing equine coronal cement, these crystals appear as small and spherical, or long and spindle-like in shape. When mature, they appear as circa 1 mm long, spindle shaped structures that are usually aligned parallel to the long axes of the cemental collagenous fibrils (Jones & Boyde 1974). Occasionally, adjacent crystals partially merge together producing an appearance similar to a string of beads (Jones and Boyde 1974). On TEM examination, human apatite crystals appear as needle-like structures of circa 60 nm long (Jones 1981).

2.7.4. Intrinsic fibrils of cement

The intrinsic fibres of cement are produced by cementoblasts and are 1-2 μm in diameter in both equine (Jones and Boyde 1974) and human (Jones 1981) cement. They are generally oriented parallel to the developing surface of cement and thus are perpendicular to the extrinsic fibres of cement (Osborn 1966; Jones and Boyde 1974). The orientation of intrinsic fibres changes in successive cement layers gives cement a lamellar appearance (Jones 1981).

2.7.5. Extrinsic fibres of cement

The extrinsic fibres are laid down by fibroblasts and anchor the periodontal ligament to the cement. They are circa 60 nm in diameter, usually forming bundles of fibres of 5-10 μm diameter that are known as the perforating fibres of Sharpey (Jones

& and Boyde 1974). Sharpey's fibres are oriented perpendicular to the developing front of human cement (Selvig 1965). In horses they follow an undulating course in cement and are oriented either obliquely or perpendicular to the developing cemental front (Jones & Boyde 1974). Microradiograpy of ground cemental sections have shown that Sharpey's fibres embedded in cellular cement had uncalcified cores of 1-5 μm diameter that are surrounded by a highly calcified peripheral zone (Selvig 1965). In fully calcified ground cemental sections, the entry holes of Sharpey's fibres into cement are readily apparent with mineralised parts of these fibres visible at their bases (Jones and Boyde 1974).

2.7.6. Incremental lines

Ground sections of cement have a lamellar appearance characterised by incremental lines running parallel to the developing front of cement. The widths of both the incremental lines and the intervals between them vary from the cementodentinal junction to the surface of cement (Selvig 1965). Incremental lines appears as virtually straight lines in LM and low magnification EM examinations, but appear as serrated lines (due to pyramidal shaped protrusion of calcified collagenous fibrils) at higher EM magnifications. The incremental lines of cellular cement are wide and intermittent (Jones 1981) and their thickness increases during rapid cemental deposition. The intrinsic fibrils are smaller in thin than in thicker incremental lines (Jones 1981).

A microradiographic study has demonstrated that incremental lines are less radiopaque in the outer cellular cement than in inner acellular cement. Additionally, incremental lines are more radiopaque near the cementodentinal junction and cemental surface than in the middle of human cement (Selvig 1965).

2.7.7. Classification of cement

As is the case for the other dental tissues, the technique for classification of cement varies between different studies. Cement is traditionally divided into acellular and cellular cement. By examination of its cellular and organic constituents, particularly its collagenous fibrils, Listgarten (1968) further classified cement into (i) cellular-fibrillar (ii) acellular-fibrillar or (iii) acellular-afibrillar. Jones (1981) however described four types of cement namely: (i) fibrillar (cellular), (ii) extrinsic fibre (acellular), (iii) mixed fibre (acellular and cellular) or (iv) intrinsic fibrils (cellular). Additionally, hypsodont cement can be divided into coronal and root cement according to its anatomical location. Coronal cement of upper cheek teeth and incisors can also be divided into peripheral and infundibular cement after the occlusal enamel is worn (fig 2).

2.7.7.1. Acellular and fibrillar cement

As acellular and afibrillar cement is the first cemental layer to be deposited in human tooth roots, it is also called primary cement (Ten Cate 1994). Histologically, this type of cement resembles the intercellular matrix of cellular cement and contains both Sharpey's fibrils radiating perpendicularly and intrinsic fibres oriented parallel to its surface (Osborn 1966; Davies 1990). Acellular cement is found on the tips of the cusps of bovine molars (Mills and Irving 1967). Listgarten (1968) proposed that acellular cement may be an equivalent to the primary cement of brachyodont teeth or that it is an acquired mineralised pellicle of connective tissue origin that developed over the exposed enamel surface prior to coronal cementogenesis.

2.7.7.2. Cellular and fibrillar cement

As the deposition of cellular and fibrillar cement follows the formation of primary cement, it is termed secondary cement by Ten Cate (1994). Immunological studies on mice teeth have indicated that the cementoblasts which form acellular cement are replaced by cementoblasts of a different phenotype prior to the formation of cellular cement (Freeman et al 1975). The cellularity of secondary cement is attributed its rapid deposition, with cementoblasts becoming entrapped in their own secretions (Ten Cate 1994). This type of cement contains cementocytes that have plum-stone shaped bodies, with numerous long processes which often radiate out towards the periodontal ligaments and also anatomose with those of adjacent cementocytes. These cells lie in lacunae which have a dark, spider-like appearance in dried cemental sections, with this dark appearance due to the presence of entrapped air in lacunae (Osborn 1966). In inner (adjacent to enamel) equine cement, these lacunae are round shaped and surrounded by a randomly oriented network of fine collagenous fibrils (Jones and Boyde 1974). The lacunae of outer (adjacent to the developing front of cement) equine cement are long and narrow in shape and their maximum lengths parallel to the developing front was 15 μm. Each lacuna has canaliculae that are 0.1-0.3 μm in diameter (Jones and Boyde 1974). In addition to the horse, cellular cement has been reported in a number of species including the elephant (Hopewell-Smith 1918), sheep (Weinreb & Sharav 1964) and rabbit (Listgarten & Kamin 1969).

2.7.7.3. Acellular and afibrillar cement

In normal human teeth, a thin radiodense layer of acellular and afibrilar cement is present near the amelocemental junction. It also is present on the occlusal

surface of teeth whose reduced enamel epithelium is absent for reasons including trauma and in the congenital disorder of amelogenesis imperfecta (Listgarten 1968; Jones 1981). Acellular and afibrillar cement is usually well mineralised and can be likened in structure to peritubular dentine or perilacunar bone (Jones 1981). The absence of fibrils in this type of cement could represent the transitory cement occurring the early stage of repair of an absorbed area (Listgarten 1968).

2.7.7.4. Coronal cement

The presence of a non calcified, membrane-like structure over the coronal enamel of both unerupted and erupted human teeth was first reported by Nasmyth (1839) and later by Owen (1845) and Tomes (1859). They considered this structure to be homologous with the coronal cement of ruminants. Tomes (1859) found no structural differences between the dental sacs of human and ruminants and claimed that this membranous structure surrounding the occlusal surface of human teeth should not to be accepted as an equivalent of the coronal cement of herbivores. Chase (1926) and later Listgarten (1968) rejected this claim by showing that the membranous structures of brachyodont teeth represented cellular structures derived from the reduced enamel epithelium of developing teeth.

The major difference between teeth with and without coronal cement is the state of preservation of the reduced enamel epithelium at the time of dental eruption, with, e.g. human teeth covered by the reduced enamel epithelial layer until dental eruption (McHuge & Zander 1965). In humans, premature degeneration of the reduced enamel epithelium, i.e. prior to tooth eruption results in the deposition of acellular-afibrillar cement in the deep enamel fissures (Kronfeld 1938; Listgarten 1968). Another example of early degeneration of the reduced enamel epithelium in

human teeth occurs in amelogenesis imperfecta, where cement is acellular and afibrillar (Listgarten 1968). However, in bovine teeth the reduced enamel epithelial layer usually disintegrates prior to eruption, thereby allowing dental follicular cells to come into direct contact with the enamel surface and consequently to be differentiated into cementoblasts and to produce coronal cement (Listgarten 1968).

The relationship between coronal cement and enamel is unclear. Glimcher et al (1964) and Levine et al (1964) claimed that in human and bovine teeth respectively, coronal cement was laid down directly on the outer surface of the reduced enamel layer, which then persists as a distinct layer between cement and enamel. On the other hand, Mills & Irving (1967) and Listgarten (1968) found that bovine coronal cement was laid down on enamel following degeneration of the reduced enamel epithelial layer. The differences between these findings may be due to either mistaking the follicular connective tissue as coronal cement (Listgarten 1968) or to loss of tissue orientation during specimen preparation (Mills and Irving 1967).

As soon as the cells of the dental follicle infiltrate the reduced enamel epithelial layer, they probably become aligned upon a basement membrane that persists between enamel and cement (Listgarten 1968; Mills & Irvings 1967). This may be one reason why cement is readily chipped from the enamel surface, but not from the dentinal surface where no such membrane exists (Mills & Irvings 1967).

The existence of coronal cement on equine molars was first reported by Havers (1691). With the use of an improved light microscope, Purkinje (1835) demonstrated the cellularity of coronal cement as well as its resemblance to bone. Owen (1845) found an irregularly pitted pattern of the amelocemental junction in

longitudinal section of equine incisors and attributed this irregularity to enamel resorption. Kawai (1955) examined ground sections of an equine molar and found resorption pits along the amelodentinal junction of equine molar that increased the contact area between enamel and coronal cement.

An SEM study of developing deciduous incisors and (permanent) molars of 4 neonatal foals by Jones and Boyde (1974) showed that coronal cementogenesis is followed by resorption of the underlying enamel surface, with a short delay occurring between the completion of enamel deposition and the beginning of enamel resorption. They believed that the purpose of this lag period was to allow the enamel surface to at least partially mature.

Jones & Boyde (1974) also found that the surfaces of resorbed equine enamel had two patterns of irregularities, the first due to difference in resorption rate between prismatic and interprismatic enamel. The second pattern, which gives a porous appearance to the resorbed enamel surface results from different rate of etching of individual or groups of crystals of prismatic and interprismatic enamel by odontoclasts. They claimed that these two patterns were similar to those produced by acid etching in laboratory conditions. These surface irregularities, including resorption bays may exist to increase the mechanical interlocking between enamel and cement (Jones & Boyde 1974).

Electron-probe radiographic analysis of equine cemental surfaces has revealed that once cement is laid down, the underlying enamel surface cannot mineralise further, because enamel maturation requires both mineral deposition as well as withdrawal of its organic matrix, conditions that cannot be achieved under cemental

cover (Jones and Boyde 1974). Listgarten (1968) reported similar findings in bovine enamel.

Coronal cement has been examined in sheep (Weinreb & Sharav 1964), cattle (Mills & Irving 1967; Listgarten 1968), the Indian elephant (Kawai 1955; Schmidt & Keil 1971) and rodents including guinea-pigs (Hunt 1959), and rabbits (Listgarten & Kamin 1969) but resorption of the enamel surface before deposition of coronal cement has only been reported in the Indian elephant (Kawai 1955; Schmidt & Keil 1971).

2.7.7.5. Root cement

In human teeth, root cementogenesis is followed by the disintegration of Hartwig' epithelial root sheath (Ten Cate 1994). In such (brachyodont) teeth, root cement encloses any dentine not encased by enamel and its thickness increases greatly from the cervical region to the apex. The surface of root cement is smooth, although occasionally, a number of shallow undulations are apparent, that are believed to be due to asynchrony at the prefunctional stage, between the rate of root growth and tooth eruption (Davies 1990). The thickness of root cement varies most in multi-rooted brachyodont teeth (Osborn 1966; Davies 1990).

CHAPTER 3. MATERIALS AND METHODS

3.1. MATERIALS

This study utilised a total of 50 teeth, including 11 upper premolar 4 (PM4), 14 upper molar 1(M1), 10 lower PM4, 11 lower M1, 2 upper and 2 lower central incisors (I1) extracted from 16 horse heads obtained from the post mortem room at the Royal (Dick) School of Veterinary Studies. The age, sexes, breeds and reason for euthanasia of these horses are recorded in **table 1.** Additionally, 4 incisors, 2 upper and 2 lower were removed from horses number 15 and 16.

Horse No	Age (years)	Sex	Breed	Reason for euthanasia	UCT		LCT	
					PM	M	PM	M
1	6	f	TB	GS	-	-	4	1
2	3	f	TB	GS	4	1	4	1
3	3	mn	ID	GS	-	-	-	1
4	10	mn	TB	GS	-	-	4	-
5	5	mn	TB	GS	-	-	-	1
6	4	f	Arab pony	GPM	4	-	-	-
7	3	mn	TB	GS	4	1	4	1
8	8	mn	TB	GS	4	1	4	1
9	18	mn	TB	GS	4	1	4	1
10	14	f	TB	Wobbler	4	1	4	1
11	9	mn	WB	GS	4	1	4	1
12	4	mn	TB	Lung abscess	-	1	-	1
13	16	f	Exmoor-x	Cushings	4	1	4	1
14	13	f	Pony-x	Cushings	4	1	4	1
15	3	f	TB	Hip fracture	4	1	4	1
16	1.5	-	Welsh pony	Pneumonia	-	1	-	1

Table 1: Details of horses utilised in dental study (f: female, mn: male neutered, TB: Thoroughbred, ID: Irish draught, WB: Warmblood, GS: Grass Sickness, GPM: Guttural pouch mycosis, UCT: upper cheek teeth, LCT: lower cheek teeth, P: Premolar, M: Molar).

3.2. METHODS

3.2.1. Extraction and sectioning teeth

After dissection of the skin, underlying connective tissues and the muscles of mastication, the maxilla and mandibulum were disarticulated at the temporomandibular joint and the dental arcades were washed. A thorough inspection

of the teeth was performed to detect any gross dental abnormalities which were recorded if present.

An upper and lower PM4 and M1 were extracted from one side of the horse's head by removing the lateral alveolar walls with a bone chisel and hammer. After fixation for a minimum of 48 hours in 10% formal saline solution, the teeth were washed thoroughly under tap water, dried in an oven at 60°C for 24 hours and then processed using one of following techniques:

(I) The teeth of horses 1 to 5 were fractured at room temperature by crushing them in a vice or by striking them with a steel hammer on a steel surface.

(II) The teeth of horses 6 to 12 were first embedded in methyl methacrylate resin (Simplex Rapid, Austenal Dental Products Ltd, Harrow, England) and then sectioned using a large industrial lathe (Colchester Master 2500, The Colchester Lathe Company Ltd., Colchester, England) and a titanium coated saw (HSS metal slitting saw, SKF & Dormer Ltd., Birmingham, England) into 8 sections, four of 1.5 mm, and four of 8 mm thick, throughout the tooth length. These sections, from the crown to apex, were termed levels (L) 1, 2, 3 and 4 (fig 7). In some older horses, where the reserve crown was worn, less than 8 sections were obtainable. The resin was removed (with difficulty) using a hacksaw, prior to decalcification or SEM examination.

(III) The teeth obtained from horses 14 to 16 were cut using a diamond tipped machine saw (Buehler model 10TS, Buehler UK Ltd., Coventry, UK, Courtesy of Mr. M. Hall, Department of Geology and Geophysics, University of Edinburgh). These teeth were sectioned in a transverse plane at the same levels and thicknesses as in method (11) above, but they were not embedded in resin prior to sectioning. This

method produced particularly good result in older teeth with small or no pulp cavities. However, as they were not protected by resin, younger teeth with larger pulp cavities tended to fracture during sectioning due to saw vibrations.

(1V) Using the previously described diamond tipped machine saw in method (III), the teeth of horse 13 were sectioned longitudinally and each half was polished utilising a self-adhesive grinding paper (P-600 grit metallographic grinding paper, Burhmer-Met, Vienna, Austria) to create flat surfaces that were mounted on frosted slides with quick setting glue ("Super glue", Loctite UK Ltd., Welwyn Garden City, UK). Finally, each half tooth was cut into 0.7 mm sections with a smaller diamond saw and a thin sectioning system (Buehler Petrothin, Buehler UK Ltd., Coventry, UK, Courtesy of Mr. M. Hall, Department of Geology and Geophysics, University of Edinburgh), which were then ground until the sections were 40-50 μm thick. Following grinding, the sections were removed from the slides and immersed in NN-Dimethyle Formamide solution (Merck Ltd., Lutterworth, UK) to remove air bubbles trapped between slides and samples. The sections were then remounted on new slides and examined under a light microscope (Orthoplan wide field light microscope, Leica UK Ltd., Milton Keynes, UK) using polarised light.

3.2.2. Preparation of specimens for light and scanning electron microscopic examinations

As noted, all upper cheek teeth were macroscopically examined for evidence of enamel or cement defects, infundibular "necrosis" (caries) or pulpar exposure. In addition, the surfaces of the 1.5 mm thick sections were photographed in order to select locations for SEM examinations. These thin sections were then decalcified for 2.5 days using Gooding and Stewart's solution (Gooding and Stewart 1932),

dehydrated through graded ethanols and cleaned with xylene. After impregnation with three changes of a combination of purified paraffin and plastic polymers ("Paraplast Plus", Sherwood Medical, Athy, Ireland), the specimens were embedded in this material. Two 10 μm thick sections were cut from each block using a microtome ('820' Spencer Microtome, American Optical Co., Buffalo, USA).

These sections were floated out on a water bath, placed on glass slides and dried overnight at 37°C. Half of these sections were stained with Hematoxylin-Eosin (H&E) to aid examination of the incremental lines of dentine, the organic constituents of tooth tissues and to allow measurement of the sizes of the cemental lacunae and vascular channels. The remaining sections were mounted on aluminium stubs (Pin Stub, Emitech Ltd., Ashford, Kent, England) using conductive carbon cement (Leit-c, Neubauer Chemikalien, Munster, Germany), dried overnight in an oven at 60°C and coated with gold-palladium (Gold/Palladium Target, Emitech Ltd., Ashford, England) in a sputter coater (SC500 Sputter Coater, Emscope laboratories Ltd., Ashford, England) (fig 7). These sections were examined in a SEM (Philips EM505, Philips Electron Optics, Eindhoven, The Netherlands) operated at 30.2 kV. This technique was particularly useful to examine odontoblast processes and the collagenous fibrils of peritubular dentine. These staining and coating methods were also useful for examination of the organic constituent of enamel, even though this organic material was largely lost during the decalcification process.

For SEM examination, the 8 mm thick blocks were cut in a vertical plane into four equal sections using a hardened blade hacksaw or the diamond saw utilised in method (II) (fig 7). Acid etching of specimens was a prerequisite to define the territories of peritubular dentine and the boundaries between enamel prisms and

interprismatic enamel. The surfaces of one of the three sections to be etched was polished utilising the previously described self-adhesive grinding paper mounted on a rotary grinder (MK 2A, Engis Ltd., Kent, England). Three of the blocks were etched in 0.005 M phosphoric acid solution for 90 seconds as described by Postek (1980) and Boyde & Martin (1982).

The sections were then washed in deionised water and dried overnight in an oven at 60°C. The fourth block was left untreated to allow microscopic comparison of untreated, with etched and decalcified (3.2.2) dental tissues. A total of 498 specimens were mounted on aluminium stubs with their upper surfaces parallel to the stub surfaces, then coated with gold palladium, followed by examination and photography in the SEM. Both the transverse and vertical surfaces of these blocks, which included enamel, dentine and cement were examined in the SEM. In order to facilitate a three dimensional examination of these specimens, sections were also rotated and tilted at various angles on the SEM stage during examination.

3.2.3. Preparation of specimens for transmission electron microscopic examination

Transmission electron microscopic examination was undertaken to investigate the crystal structure of enamel and the soft tissue constituents of dentine and cement. Some TEM examinations were performed on sections which had previously been examined by SEM or light microscopy. Undecalcified sections from the SEM study were partially decalcified in 10% EDTA (Ethylene Diamine Tetraacetate, BDH Chemicals Ltd., Poole, England) solution at PH 7 at 20°C for 72 hr, then fixed in 3% gluteraldehyde in 0.1M sodium cacodylate buffer pH 7.3 for 2.5 hr, followed by washing in three changes of 0.1 sodium cacodylate buffer for 1 hr.

After postfixation with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for 45 minutes, the specimens were washed in distilled water for 20 minutes on three occasions, dehydrated in a graded series of acetone and infiltrated in a mixture of araldite CY212 (Agar Scientific Ltd., Stansted, UK) and dodecenly succinic anhydride (Agar Scientific Ltd., Stansted, UK) (Sabatini et al 1962).

At the final stage of infiltration and embedding, an accelerator containing 67% Dibutylpthalate (Agar Scientific Ltd., Stansted, UK) and 33% Dimethylaminomethyle (DMP)-30 (Agar Scientific Ltd., Stansted, UK) was added to the araldite and DDSA mixture to harden the samples. One µm thick specimens were then cut on an ultramicrotome (Reichert Ultramicrotome OMU4, Leica UK Ltd., Milton Keynes, UK) utilising a glass knife. Sections were then stained with toluidine blue and examined under the LM to select appropriate areas to obtain sections for TEM examination. Finally, using the above microtome, 60 nm thick sections were cut and mounted on copper grids, stained with uranyl acetate and lead citrate (LKB Ultrastainer, Leica UK Ltd., Milton Keynes, UK) and examined in a TEM (Philips EM400, Philips Electron Optics, Eindhoven, The Netherlands).

3.3. Examination of enamel

Pilot studies showed that equine cheek teeth contained two main types of enamel one adjacent to the amelodentinal junction and one adjacent to the amelocemental junction that were termed equine types 1 and 2 enamel respectively. To avoid confusion with previous classifications such as Boyde's enamel types (1964) or Koenigswald & Clemens's (1992) enamel classification, the term equine (eq.) enamel type will be used in this study. In order to quantify the structural components of these two main enamel types, the diameter of enamel prisms and the

interprismatic distances were measured at vertical levels 1, 2 and 3 of an upper and lower PM4 of three horses (18 specimens). After fixing the SEM magnification at x3860, two locations were selected for the measurement of eq. types 1 and 2 enamel respectively, one circa 10 μm from the amelodentinal junction and the other, adjacent to the amelocemental junction (figs 2 & 3).

The diameter of 9 individual prisms and the interprismatic distances at 9 different areas in each of the 18 sections were drawn on acetate sheets covering the SEM screen and were later measured using a videoplan (Mop-Videoplan, Kontron Electronic Ltd., Watford, England) and the data were recorded on both the "Microsoft excel V.5.0" computer package (Microsoft Corporation, Washington, USA) and "Minitab for Window" computer package (Minitab Inc., Philadelphia, USA). Statistical comparisons of these two variables were made between individual horses, different teeth and different vertical levels, different types of enamel, level and tooth, type and tooth, and level, type and tooth.

Eighteen locations on the surface (transverse plane) of enamel folds of the upper cheek teeth, including peripheral and infundibular enamel, and 12 locations on (peripheral) enamel of the lower cheek teeth were selected for measuring the distribution of eq. types 1 and 2 enamel. These measurements were made at vertical level 2 of PM4 in the three horses. Overall enamel thickness was measured at the same locations at levels 1, 2 and 3 of PM4 of four horses. As the infundibular enamel had either disappeared or was scant at vertical level 4 (apical region of reserve crown), enamel measurements were not performed at this level. The linear thickness of enamel was drawn on acetate sheets at fixed SEM magnification (x15.1) for all

specimens and were measured and recorded using a videoplan and the previously described computer programmes.

To analyse the distribution of eq. types 1 and 2 enamel, the boundaries of the amelodentinal and amelocemental junctions and the transitional zone between eq. types 1 and 2 enamel were drawn on acetate sheets which were also analysed by a videoplan to determine the proportions of the two enamels. Finally photographs of the transverse surfaces of the upper and lower PM4 were scanned into a computer (Macintosh IISi, Apple Computer Limited, Hemel Hempstead, England) using a computer package (Aldus freehand V.3.1, Aldus Europe Limited, Edinburgh, Scotland). This procedure enabled maps of the enamel to be drawn and the distribution of the enamel types to be superimposed on the diagrams.

3.4. Examination of dentine

Pilot SEM studies showed that in horses, the number and diameter of dentinal tubules, the diameter of peritubular dentine and the amount of intratubular dentine varied greatly from the amelodentinal junction towards the pulp cavity. Four locations were selected at random in the middle of primary dentine in untreated and etched dentinal sections at a fixed SEM magnification (x2020) to assess the number of dentinal tubules per unit area. Identical locations were selected for vertical levels 1, 2, 3 and 4, in an upper and lower M1 of 8 horses (16 teeth, 64 specimens, 136 examination sites). Using a standardised magnification (x2020) for all specimens, the dentinal tubules were outlined with a fine felt tipped pen on acetate sheets covering the SEM screen and this allowed the number of dentinal tubules in a known surface area to be counted.

Three locations; (1) in primary dentine, 40 μm from the amelodentinal junction, (2) in primary dentine adjacent to the border with secondary dentine (3) within secondary dentine, were selected for measurements of the diameters of dentinal tubules and the areas and diameters of peritubular dentine (figs 2 & 3). Identical locations were utilised at all four vertical levels of the 16 teeth. Magnification was again fixed at x2020 for all specimens and the boundaries of the tubules and peritubular dentine were traced on acetate sheets that were later examined in the videoplan. This allowed the diameter of dentinal tubules and both the surface area and diameters of peritubular dentine to be measured. The transverse area of intertubular dentine was calculated by deducting the area of peritubular dentine from the total dentinal area. Results of the numerical density and diameter of dentinal tubules, the area and diameter of peritubular dentine and the amount of intertubular dentine were recorded on the previously described computer programmes. Comparisons of these variables between the different dental vertical levels, regions (locations), teeth (upper and lower), horses, regions and teeth and regions and levels were made.

3.5. Examination of cement

When the structure of cement was studied, particular attention was paid to cement adjacent to the amelocemental junction. The quantitative cemental measurements undertaken, included the sizes and numerical density of lacunae and the ratio of lacunae volume: total cement volume. These measurements were made at two random locations, one in infundibular and one in peripheral cement of upper PM4s of 7 horses, and in a single random location in the peripheral cement of lower PM4s of 7 horses. A 4 mm side graticule (Square Graticule, Graticules Ltd., Kent.

England), was placed in the light microscope in order to select locations for examination. The sizes and diameters of lacunae within the graticules were measured using the videoplan and data were recorded on the previously described computer programmes. Statistical comparisons were performed between infundibular and peripheral cement of the upper cheek teeth, cement at different vertical levels of the same teeth, the upper and lower teeth of the same horses (peripheral cement) and between different horses.

3.6. Statistical techniques

When data were normally distributed they are presented as mean and standard deviation (± SD) or mean and standard error of mean (SM) and were analysed using parametric techniques, i.e. analysis of variance (ANOVA). When data were non-normally distributed they are presented as median and range and all statistical comparison on such data were performed utilising non-parametric techniques, i.e. the Mann-Whitney test to compare two groups and Kruskal-Wallis test for comparing more than two groups. All calculations were performed using a computerised statistical package "Genstat-5 (Rothamsted Experimental Station) (Payne 1987).

CHAPTER 4

GROSS, LIGHT MICROSCOPIC AND ULTRASTRUCTURAL EXAMINATIONS OF ENAMEL

RESULTS

4.1. Gross appearances of cheek teeth

In the SEM, the surface of occlusal enamel was uneven and contained many pits of irregular sizes and shapes (fig 8). The occlusal surface of untreated enamel in some horses was covered by an organic pellicle of variable thickness that contained different sized holes (fig 9). On the occlusal surface of teeth, the exposed dentine showed depressions extending from the amelodentinal junction towards the secondary dentine (at the site of the obliterated pulp cavities). The depth of these depressions was related to the area of the occlusal surface of dentine, with larger dentinal surface areas having deeper depressions.

On transverse sections of cheek teeth, both peripheral and infundibular enamel had very irregular courses, with many invaginations into the neighbouring dentine. A section of an upper PM4 (fig 10) shows the distribution of the different dental tissues. On transverse section, the gross configurations of dental tissues in upper PM4 were similar to those of upper M1, but examinations of 7 PM4s and 9 M1s showed that the occlusal surfaces of upper PM4 were larger and more rhomboidal in shape than those of upper M1, which were smaller and squarer in outline. On transverse section, both upper PM4 and M1 contained a deep ridge of enamel towards the lingocaudal angle of the mesial infundibulum (fig 10) and this invagination was deeper and smoother in PM4 than in M1. On transverse section, the mesiobuccal angle of peripheral enamel of both upper PM4 and M1 formed a

caudally curved, hook like structure, that was longer and more caudally curved in PM4 (fig 10).

Examination of transverse sections of 18 upper and 19 lower teeth showed that the lower cheek teeth were smaller and more rectangular in shape than their upper counterparts (figs 10 & 13). Although the lower cheek teeth contained no infundibula, the caudolingual enamel invagination fully merged in some sections to form an infundibulum-like cemental lake towards the tooth apex (vertical levels 3 and 4) (fig 11).

4.2. Enamel thickness

Linear measurement of transversely sectioned enamel of 4 upper PM4s showed that both peripheral and infundibular enamels were approximately three times thicker in areas where they were parallel to the long axis of the maxilla (median 1.07 mm, 0.7-1.45) than in areas where they were perpendicular to this axis (median 0.36 mm, 0.21-1.16). These figures also demonstrate that variation in enamel thickness is greatest in areas that are perpendicular to the long axis of the maxilla. The buccal (lateral) aspects of both peripheral and infundibular enamel (median 1.23 mm, range 0.78-1.45) were thicker than their palatal (medial) counterparts (median 0.78 mm, range 0.7-1.17). The caudal aspect of the mesial infundibular enamel was the thinnest enamel of the upper cheek teeth with a median thickness of 2.01 mm (range 0.15-0.32) for upper PM4. This mesial infundibular enamel also showed several sharp changes of direction (fig 12), however these features disappeared towards the apex of the infundibulum (vertical level 4).

Examination of mandibular cheek teeth showed that after the secondary occlusal surface had formed, no major differences were present in enamel

configuration between lower PM4 and M1, with both having irregular folds of enamel situated between peripheral cement and dentine (fig 13). As was the case with the maxillary teeth, the deeply folded peripheral enamel of the mandibular cheek teeth was thicker when it was parallel to the long axis of mandibulum (median 0.79 mm, range 0.33-1.03) than where perpendicular to this axis (median 0.29 mm, range 0.22-0.49). Enamel was thinner in deeply invaginated areas (median 0.42 mm, range 0.22-0.82), than in the remaining regions (median 0.91 mm, range 0.65-1.03). In total contrast to the upper cheek teeth, lower cheek teeth enamel was thicker at the buccal (lateral) aspects (median 1.02 mm, range 0.22-1.03) than at the lingual (medial) aspects, (median 0.79 mm, range 0.65-0.91).

Statistical analyses of enamel thickness showed data to be normally distributed and data are presented as mean (±SD).

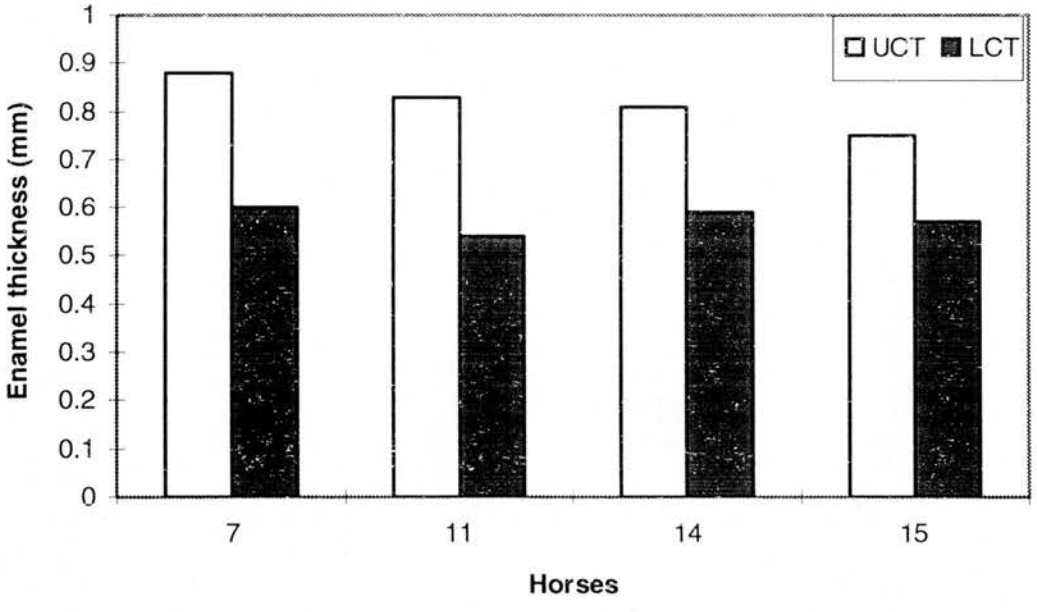

Fig 14: Mean enamel thickness (mm) of 4 upper cheek teeth (UCT) and 4 lower cheek teeth (LCT) of 4 individual horses.

The mean (± SD) enamel thicknesses of the 3 vertical levels at 18 locations of upper and 12 locations of lower PM4 of 4 individual horses are given respectively in appendixes 1 & 2.

For both upper and lower cheek teeth, a highly significant ($p< 0.001$) difference existed for location on a transverse plane, but for any individual site in the transverse plane no significant difference existed between the 3 vertical levels, indicating that the depth into the tooth has no influence on this parameters (**tables 2 & 3**).

Source of variation	**Degree of freedom**	**Sum squares**	**Mean squares**	**F Ratio**	**Probability**
Level	2	560	280	0.57	0.583
Error	9	4396	488		
Location	17	313464	18439	274	< 0.001
Level x Location	34	1709	50.3	0.75	0.840
Error	153	10298	67.3		
Total	215	330427			

Table 2: Analysis of variance of effects of level (vertical) and location (transverse) on overall enamel thickness (µm) of upper PM4 in 4 horses

Source of variation	**Degree of freedom**	**Sum squares**	**Mean squares**	**F Ratio**	**Probability**
Level	2	92.1	46.05	0.29	0.758
Error	9	1452	161.3		
Location	11	111802	10164	232.5	< 0.001
Level x Location	22	389	17.7	0.40	0.991
Error	99	4328	43.72		
Total	143	118062			

Table 3: Analysis of variance of effects of level (vertical) and location (transverse) on overall enamel thickness (µm) of lower PM4 in 4 horses

The results of these measurements also showed that the range of enamel thickness were significantly greater in the upper (0.15-1.62 mm) than in the lower (0.18-1.2 mm) cheek teeth.

4.3. Ultrastructure of equine cheek teeth enamel

Enamel types were classified according to the appearances of their prisms in the transverse plane (cross-section) and the amount and appearance of interprismatic enamel they contained. In addition to the two main types of enamel, termed eq. types 1 and 2, smaller quantities of a further morphologically distinct enamel type that was termed eq. type 3 enamel were also identified on SEM examination.

4.3.1. Equine type 1 enamel

Eq. type 1 enamel was located at the amelodentinal junction (medially), and in the upper cheek teeth where enamel was widest, this type of enamel extended for approximately two-thirds of the enamel thickness. Eq. type 1 enamel had enamel prisms that were almost oval on cross section and were organised in parallel rows, with rows of prisms alternating with flat plates of interprismatic enamel (fig 15). Some rows followed a straight course to the amelocemental junction while others deviated to the left or right (figs 15, 16 & 17). At higher magnification (x2720), the enamel prisms were shown to be of slightly variable diameters and slightly differing distances from each other (fig 16). The proportions of interprismatic enamel to prismatic enamel also varied, depending on the location of the enamel (figs 15, 17 & 18).

4.3.2. Equine type 2 enamel

Eq. type 2 enamel contained no interprismatic enamel plates (figs 18, 19 & 20) but solely consisted of individual prisms, separated by spaces of variable thickness that appeared dark on acid etched sections on SEM examination (figs 19 & 20) and pale on TEM examinations of partially decalcified sections (fig 21).

These spaces were believed to contain little or no calcified material. In any one area, eq. type 2 prisms were very similar on transverse section (figs 19 & 20), but in different regions of the enamel, these profiles ranged from circular (fig 18) to "keyhole" or "horseshoe" shape, largely depending on the angle between the long axes of the prisms and the direction of specimen sectioning (fig 18). On TEM examination, the "keyhole" pattern was more obvious when sections were cut oblique to the long axes of prisms (fig 21). The "horseshoe" shaped prisms were arranged in columns, with the open aspect of a "horseshoe" accommodating the closed aspect of an adjacent prism (fig 20 & 21). In other areas, particularly where the prisms were circular in outline, they were arranged in orderly rows (fig 22). SEM examination of both acid etched and untreated sections showed the transitional zone between eq. types 1 and 2 enamel in both upper and lower cheek teeth to be very irregular (figs 18 & 23).

4.3.3. Equine type 3 enamel

Eq. type 3 enamel was composed of prisms that were completely surrounded by large quantities of interprismatic material (figs 18 & 24). The interprismatic material formed a honeycomb like structure, with each "cell" occupied by a prism that was oval on cross section (fig 24). This type of enamel was inconsistently found in a thin layer (median 9.51 μm, range 5.04-15.65 thick) at both the amelodentinal (fig 18) and amelocemental junctions (fig 24). At the amelodentinal junction, eq. type 3 interprismatic enamel merged with the interprismatic plates of eq. type 1 enamel (fig 18) whilst at the amelocemental junction, eq. type 3 enamel merged with eq. type 2 enamel (fig 24).

At the amelocemental junction, the distal 2-3 μm aspects of equine type 2 enamel prisms tapered to a conical shape which was of similar appearance to the amelodentinal (initial) aspects of equine type 1 prisms (fig 25). Where enamel changed direction at macroscopic folds, there was much variation in prism diameter. In both eq. types 1 and 2 enamel, individual prisms showed variation in thickness along their lengths (fig 26). Polarised light microscopy of ground enamel sections showed that the irregular incremental lines of enamel were formed from rows of dilatations and constrictions of adjacent prisms.

4.4 Measurements of prism diameters and interprismatic distances in eq. types 1 and 2 enamel

As noted (3.3), prism diameters and the distance between enamel prisms, i.e. interprismatic distances, were measured at two locations, i.e. adjacent to the amelodentinal and amelocemental junctions and at three vertical levels (1-3). At each location, the diameter of 9 individual prisms and the interprismatic distance at 9 different regions were measured for both eq. types 1 and 2 enamel. These data were normally distributed and results are presented as mean (±SD) and were analysed using analysis of variance.

The combined values of prism thickness and interprismatic distances of eq. types 1 and 2 in upper and lower PM4s of 3 horses are presented in **tables 4 and 5**. Measurements of prism diameter and interprismatic distances of the individual horses are presented in appendices 3, 4, 5 & 6.

Teeth	Levels	Prism diameter		Interprismatic distance	
		Mean	SD	Mean	SD
Upper	1	2.06	1.04	1.63	0.42
	2	2.35	0.79	1.99	0.81
	3	1.68	0.43	1.85	0.53
Lower	1	2.06	0.74	2.16	0.74
	2	2.16	0.89	.59	0.36
	3	1.80	0.53	1.74	0.49

Table 4: Prism diameter and interprismatic distance (μm) [mean and standard deviation (SD)] of equine type 1 enamel at 3 different vertical levels in upper and lower PM4 of 3 individual horses

Teeth	Levels	Prism diameter		Interprismatic distance	
		Mean	SD	Mean	SD
Upper	1	3.60	1.08	0.61	0.18
	2	4.65	0.95	0.65	0.11
	3	3.92	1.35	0.72	0.39
Lower	1	3.17	1.15	0.60	0.28
	2	3.71	1.07	0.60	0.23
	3	4.28	1.32	0.77	0.40

Table 5: Prism diameter and interprismatic distance (μm) [mean and standard deviation (SD)] of equine type 2 enamel at 3 different vertical levels in upper and lower PM4 of 3 individual horses

Analysis of variance on these data showed that variation in prism diameters was highly significant ($p< 0.001$) between eq. types 1 and 2 enamel and was also highly significant ($p< 0.001$) both between vertical levels 1, 2 and 3 of the same type of enamel and between the upper and lower cheek teeth **(table 6).** These results indicate that prism diameters significantly increase in size from the amelodentinal junction to the amelocemental and that there are significant differences between diameters of prisms at different depths of the tooth and also between the upper and lower cheek teeth.

Source of variation	Degree of freedom	Sum squares	Mean squares	F Ratio	Probability
Horse	2	51.72	25.85		
Level (L)	2	13.23	6.614	1.71	0.290
Error	4	15.45	3.862		
Type (t)	1	283.56	283.56	273.35	0.004
Error	2	2.07	1.037		
Tooth (T)	1	2.50	2.50	0.07	0.81
Error	2	66.77	33.38		
L x t	2	14.5916	7.2958	11.76	< 0.001
L x T	2	8.9215	4.4607	7.19	< 0.001
t x T	1	1.9519	1.9519	3.15	0.078
L x t x T	2	3.4480	1.7229	2.78	0.065
Error	190	117.85	0.6203		

Table 6: Analysis of variance of effect of vertical level, enamel type and different teeth on prism diameter (µm) of upper and lower premolar 4 in 3 individual horses.

Analysis of variance of interprismatic distances showed no significant variation ($p< 0.136$) between enamel types, vertical levels or between upper and lower teeth, but significant variation ($p< 0.001$) existed within each enamel type at different vertical levels of the teeth **(table 7).**

Source of variation	Degree of freedom	Sum squares	Mean squares	F Ratio	Probability
Horse	2	4.2422	2.1711		
Level (L)	2	0.0718	0.0359	0.07	0.931
Error	4	1.9732	0.4933		
Type (t)	1	107.25	107.25	160.31	0.006
Error	2	1.3381	0.6691		
Tooth (T)	1	0.0332	0.0332	0.04	0.856
Error	2	1.5801	0.7901		
L x t	2	0.8736	0.4368	2.03	0.136
L x T	2	2.5466	1.2733	5.93	0.003
t x T	1	0.02355	0.11	0.741	
L x t x T	2	3.7068	1.8534	8.63	< 0.001
Error		40.7885	0.2147		

Table 7: Analysis of variance of effect of vertical level, enamel type and different teeth on interprismatic distance (µm) of upper and lower premolar 4 in 3 individual horses

The ratio of prism diameter:interprismatic distance was 1.11 in eq. type 1 and 5.89 in eq. type 2 enamel in the upper cheek teeth. The corresponding values were 1.09 and 5.35, respectively for the lower cheek teeth.

The results of measurements of the diameter of prisms and interprismatic distance are summarised in fig 27, which shows that the diameter of eq. type 1 enamel prisms is greater in upper than in lower cheek teeth. Although non significantly different, the interprismatic distance of eq. type 1 enamel appears to be much greater than the interprismatic distance of eq. type 2 enamel. Additionally, this figure shows that in eq. type 1 enamel, the diameter of the enamel prisms is very similar to the thickness of the interprismatic enamel.

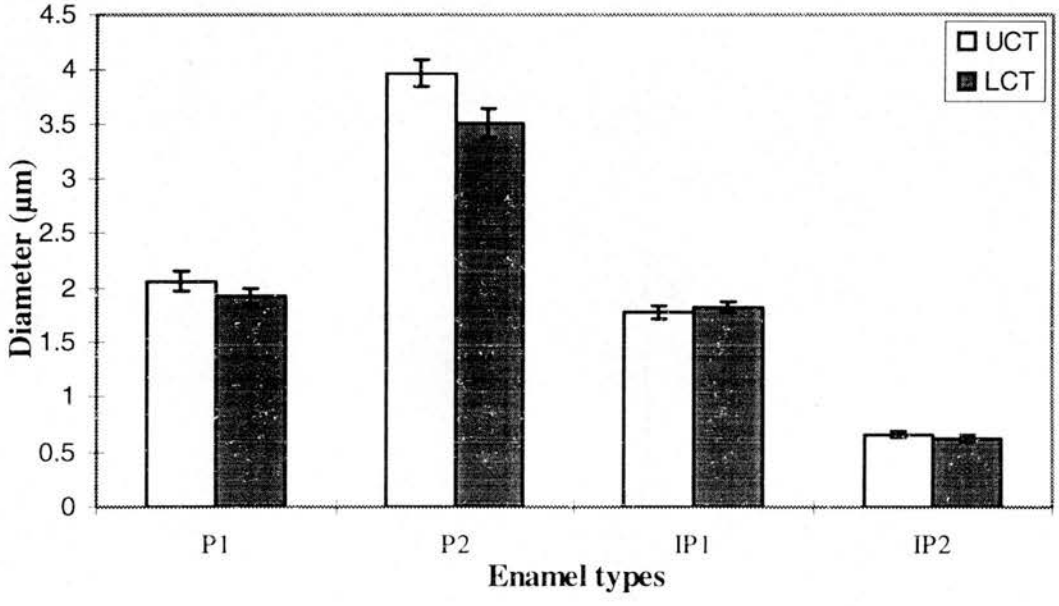

Fig 27: Prism diameters and interprismatic distances of upper cheek teeth (UCT) and lower cheek teeth (LCT). P1 (prisms of eq. type 1 enamel); P2 (prisms of eq. type 2 enamel); IP1 (interprismatic distance in eq. type 1 enamel); IP2 (interprismatic distance in eq. type 2 enamel).

4.5. Orientation of eq. types 1 and 2 enamel prisms

Eq. type 1 enamel prisms were oriented at an angle of approximately 45° to both the amelodentinal junction and the occlusal surface of the tooth, but eq. type 2 enamel prisms were orientated at a wide variety of oblique angles to both the amelodentinal junction and the occlusal surface (fig 28). In contrast to the orderly prism arrangement of eq. type 1 enamel, the arrangement of prism rows in eq. type

2 enamel varied greatly. Eq. type 2 enamel prisms originating from relatively straight parts of the amelodentinal junction, such as in the thick buccolingual enamel of the lower cheek teeth followed almost straight parallel courses and formed wide oblique angles to the amelocemental junction (figs 20 & 26). On transverse sections, prisms originating from gross enamel ridges deflected at different angles, to the right and left.

4.5. Proportions and distribution of eq. types 1 and 2 enamel

The mean amounts of eq. types 1 & 2 enamel at 18 location of upper PM4 of 3 individual horses are given in fig 29 and individual measurements are given in appendix 7. For clarity, the thin and inconsistent layers of eq. type 3 enamel present at the amelodentinal and amelocemental junctions are not shown in fig 29. These data were normally distributed and they were analysed using analysis of variance. Statistical analyses of these data showed that the overall amounts of eq. types 1 and 2 enamel significantly differed ($p < 0.001$) between various locations on transverse sections of upper PM4 **(table 8).**

Source of variation	Degree of freedom	Sum squares	Mean squares	F Ratio	Probability
Horses	2	58.92	29.46		
Location	17	60849	3579	127	< 0.001
Type (t)	1	16533	16533	588	< 0.001
t x location	15	4868	324	11.55	< 0.001
Error	66	1855	28.11		

Table 8: Analysis of variance on proportions of equine types 1 and 2 enamel at 18 locations on the transverse plane in upper PM4s of 3 horses.

The overall amounts of eq. types 1 & 2 enamel at 12 locations on a transverse section of lower PM4 of 3 individual horses are given in fig 30 and individual measurements are presented in appendix 8. For clarity, the thin and

inconsistent layers of eq. type 3 enamel present at the amelodentinal and amelocemental junctions are not shown in fig 30. The amounts of eq. types 1 and 2 enamel in lower PM4 significantly differed ($p< 0.001$) between all locations and the proportion of eq. types 1 and 2 enamel also differed significantly ($p< 0.118$) throughout the transverse locations (**table 9**).

Source of variation	Degree of freedom	Sum squares	Mean squares	F Ratio	Probability
Horses	2	294	147		
Location	11	20149	1832	12.8	< 0.001
Type (t)	1	363	363	2.54	0.118
t x location	11	3686	335	2.35	0.022
Error	46	6565	142.7		

Table 9: Analysis of variance on proportions of equine types 1 and 2 enamel at 12 locations in the transverse plane in lower PM4s of 3 horses.

Eq. type 2 enamel increased in thickness in the peripheral enamel folds (ridges), and decreased where the folds invaginated towards the centre of the tooth (fig 30). The only peripheral enamel not containing some eq. type 1 enamel was the caudal peripheral enamel of the upper cheek teeth (fig 29). In contrast, the caudal face of the mesial infundibulum contained only eq. type 1 enamel (fig 29). The distribution of eq. types 1 and 2 enamel in upper and lower cheek teeth is shown in figs 29 & 30.

There were certain similarities between the distribution of enamel types in the upper and lower cheek teeth (figs 29 & 30). At the amelodentinal junctions, eq. type 1 enamel was present, whilst at the amelocemental junction eq. type 2 enamel was present. The greatest difference in enamel type between upper and lower cheek teeth was that the largest component of peripheral enamel of the upper teeth was eq. type 1 (fig 31), as compared to eq. type 2 in the lower teeth (fig 32).

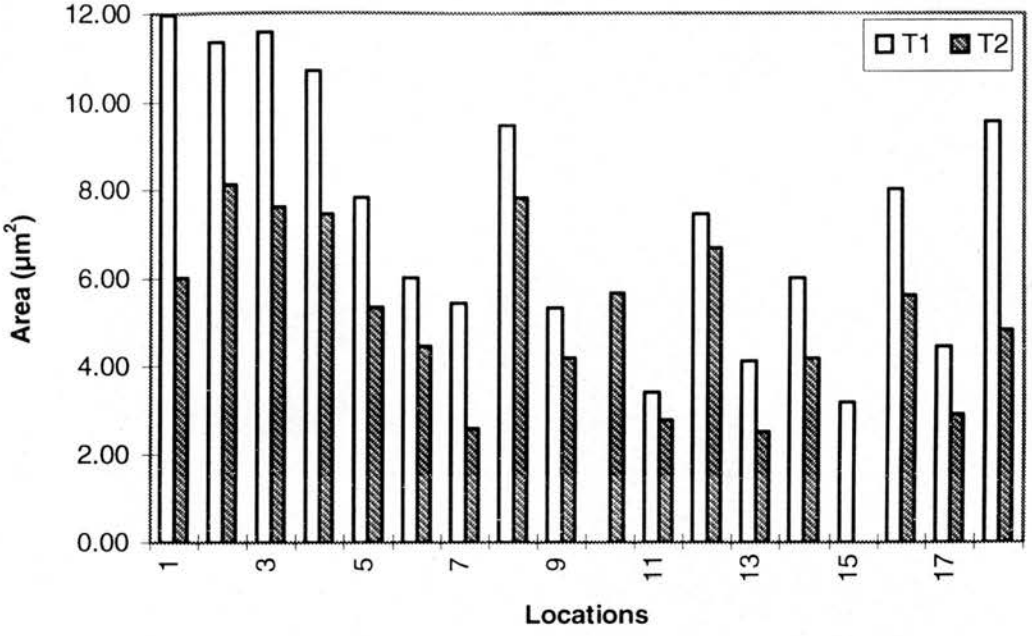

Fig 31: Mean surface area (mm^2) of equine type 1 (T1) and type 2 (T2) enamel in 18 locations of upper PM4 of 4 horses (see fig 30).

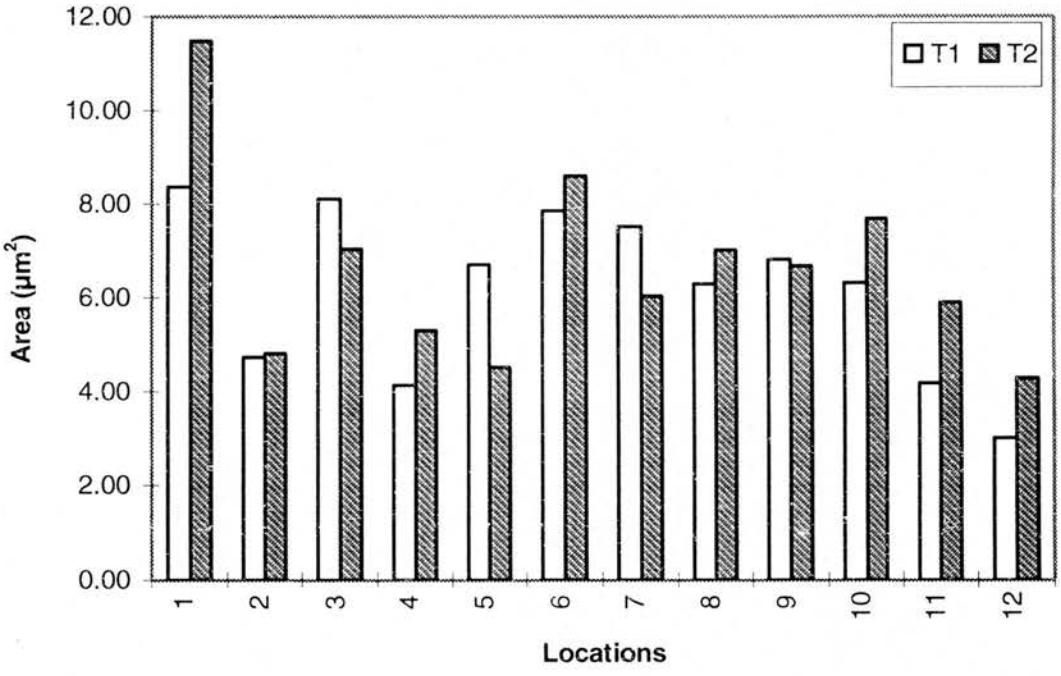

Fig 32: Mean surface area (mm^2) of equine type 1 (T1) and type 2 (T2) enamel in 12 locations of lower PM4 of 4 horses (see fig 31).

4.6. Hunter-Schreger bands (diazone and parazone)

Low power SEM examination of vertical sections of peripheral enamel, of upper cheek teeth in particular, showed the thickest buccolingual aspect to have alternating bands of transverse (diazone) and vertical (parazone) prisms (Hunter-Schreger [H-S] bands) present from crown to apex (fig 33). These bands extended from adjacent to the junction of eq. types 1 and 2 enamel to the amelocemental junction, i.e. were within type 2 enamel and were oriented perpendicular to the amelocemental junction (i.e. horizontal to the occlusal surface) (fig 33).

Examination of unetched ground enamel sections with a polarised light microscope showed that in vertical sections, this enamel layer appeared dark or white, depending on the degree of specimen rotation (figs 34 & 35). However, polarised light examination of undecalcified ground sections of peripheral upper cheek teeth enamel showed that eq. type 2 enamel contained alternating white and dark bands, similarly oriented to the above noted diazone and parazone bands (figs 34 & 35). When specimens were rotated during examination, the white bands became dark and the dark bands become white. Additionally, the infundibular enamel of upper and the peripheral enamel of lower cheek teeth exhibited an irregular, thick dark band located at the transitional zone of eq. types 1 and 2 enamel that was aligned parallel to the amelodentinal junction (fig 36). With specimen rotation, this irregular band either disappeared or moved towards the amelodentinal or amelocemental junctions.

4.7. Enamel crystals

SEM examination showed that enamel prisms were formed by parallel bundles of crystals that were almost cylindrical on transverse section (figs 19, 20 &

22). In eq. types 1 and 3 enamel, the prism crystals were oriented vertical to the crystals of interprismatic enamel thus creating spaces of variable thickness between the prismatic and interprismatic enamels. TEM examinations showed great variation in the shapes and arrangements of prismatic enamel crystals as compared to interprismatic enamel crystals. In transverse section prismatic crystals had shapes, ranging from near oval to rectangular and formed small subunits, with crystals diverging from each other at various angles (fig 37). Rectangular shaped crystals were 12 to 34 nm wide (median 24.14) and 33-94 nm deep (median 60 nm). As most sections were cut transverse to the long axes of enamel prisms, it was not possible to measure crystal lengths.

Some prisms contained empty spaces that were surrounded by bands of organic tissue. The outlines of these spaces were similar to those of crystals, i.e. on transverse section they had shapes that ranged from oval or to rectangular (fig 38). Interprismatic enamel crystals usually followed a straight parallel courses, with a minority having a wavy path (fig 39). Measurements of longitudinally sectioned interprismatic crystals showed them to be 14 to 55 nm wide (median 25.29 nm), but as previously noted, the full lengths of interprismatic enamel crystals were not visible and therefore crystal length could not be measured.

4.8. Incisor enamel

Ultrastructural examination of 2 upper and 2 lower incisors also showed three enamel types, distributed in a similar pattern to cheek teeth enamel, i.e. eq. type 1 formed the internal, type 2 the external enamel layer and type 3 was inconsistently present as a thin layer along the amelodentinal and amelocemental junctions. Measurements of prism diameter and interprismatic distance of incisor

teeth in 2 horses were performed adjacent to amelodentinal and amelocemental junctions at two vertical levels (1 and 2) and data are presented in appendixes 9, 10, 11 and 12.

Eq. type 1 incisor enamel was found to contain prisms with a mean diameter of 3.60 ± 1.20 μm in the upper and 4.23 ± 1.74 μm in the lower incisors. As was the case in the cheek teeth, these prisms were aligned in parallel rows that were separated by thick plates of interprismatic enamel. The mean thickness of interprismatic enamel was 1.99 ± 0.54 μm and 1.76 ± 0.49 μm in the upper and lower incisors, respectively. The mean diameter of eq. type 2 enamel prisms was 4.59 ± 1.166 μm in the upper and 5.71 ± 2.50 μm in the lower incisors. In the incisors, eq. type 2 enamel prisms were significantly larger than eq. type 1 enamel prisms. In contrast to cheek teeth enamel, eq. type 2 enamel prisms in incisor teeth were separated from each other by a thin layer of interprismatic enamel which was largely removed during acid etching. The mean thickness of the residual interprismatic enamel spaces was 0.82 ± 0.20 μm in the upper and 0.72 ± 0.20 μm in the lower incisors. A comparison of cheek teeth and incisor enamel prisms diameters and interprismatic distances is presented in fig 42

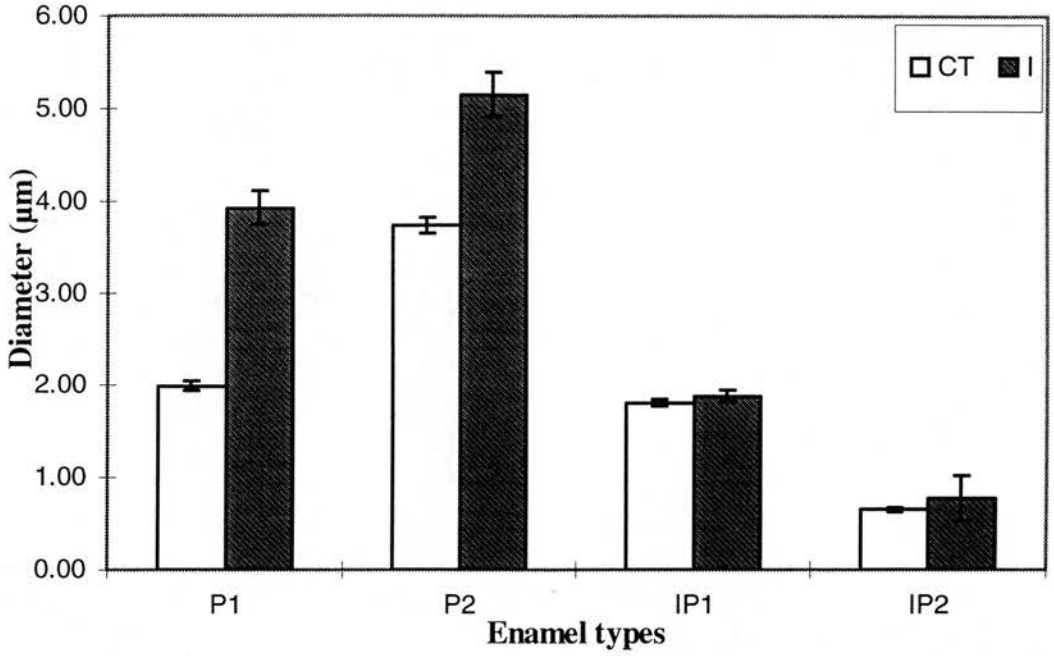

Fig 42: Prism diameter and interprismatic distance of cheek teeth (CT) and incisors (I). P1(prisms of eq. type 1 enamel); P2 (prisms of eq. type 2 enamel); IP1 (interprismatic distance of eq. type 1 enamel); IP2 (interprismatic distance of eq. type 2 enamel).

In incisor eq. type 1 enamel, interprismatic enamel plates frequently adjoined with each other and this branching pattern prevented the rows of prisms from following consistent radial courses (fig 40), but the mesial aspects of both peripheral and infundibular enamel were exceptions, and in these areas, the prism rows followed a regular, although wavy courses. Eq. type 2 enamel dominated incisor enamel and contained of bands of prisms that were aligned vertically, obliquely or even horizontally to the occlusal surface (fig 41). The apical aspects of incisor infundibular enamel contained only eq. type 2 enamel.

Examination of the labial aspects of polished incisor enamel surfaces showed a series of ridges divided by narrow grooves of constant thickness, oriented perpendicular to the amelocemental junction. On SEM examination of vertical sections, incisor enamel contained consecutive transversely and vertically oriented

prism bands, at circa 10 μm intervals, extending from crown to apex. These bands extended perpendicularly from the boundaries of eq. types 1 and 2 enamel to the amelocemental junction (fig 43).

In summary, the main differences between incisor and cheek teeth enamel were the presence of increased proportions of eq. type 2 as compared to eq. type 1 enamel in cheek teeth, and more complex orientation of prisms and prism rows within each type as compared to cheek teeth enamel. Incisor eq. type 2 enamel prisms definitely contained interprismatic enamel. Additionally, the mean prism thickness of both eq. types 1 and 2 enamel were significantly greater in incisors than in cheek teeth (fig 42).

4.2. DISCUSSION

4.2.1. Enamel folds

The presence of extensive gross folding of both peripheral and infundibular enamel not only increases the amounts of occlusal enamel but also increases the area of contact between enamel and the neighbouring cement and dentine. In addition to this large contact area between enamel and the two other calcified dental tissues at this macroscopic levels, SEM examination showed numerous microscopic pits and ridges on the dentinal and cemental interfaces of enamel, more pronounced and irregular at the latter, which further increases the contact areas between enamel and the other dental tissues and these will be described in greater detail in chapters 5, 6 and 7.

At the amelodentinal junction, the crystals of enamel and dentine merge with each other and this further increases contact between these two tissues, without the need for an excessively irregular interface. Because cement is laid down on the surface of fully formed enamel, the cement crystals cannot therefore merge with those of enamel, as occurs between dentinal and enamel crystals. Therefore, the relationship between cement and enamel depends fully on connections between macroscopic and microscopic ridges rather than at the ultrastructural level between their crystals. This may explain why the cemental interface of equine enamel is more irregular than its dentinal interface. In conclusion, equine enamel has extensive, gross and ultrastructural connections with the adjacent calcified dental tissues which should allow these tissues to effectively share the forces of mastication.

As noted the occlusal dentine surface contained depressions whose depth was related to the surface area of dentine, with larger areas having deeper depressions.

This is a result of the higher occlusal wear rate of the relatively soft dentine as compared to enamel. As the shape of the dentinal occlusal surface area is defined by the adjacent enamel folds, the orientation and invaginations of the enamel folds play an important role in reducing the occlusal surface area of dentine. If enamel was confined to the periphery of large hypsodont teeth as is the case with in brachyodont teeth, attrition would cause a very deep depression in the larger dentinal occlusal surface and this might render the tooth liable to fracture. As the exposed dentinal surface of hypsodont teeth is reduced and compartmentalised by enamel folds and invaginations, it is therefore less affected by attrition. The presence of the two infundibula in the upper cheek teeth of equidae further reduces and divides the occlusal dentinal areas.

In contrast, the lower cheek teeth have three deep peripheral enamel infoldings, one on the buccal and two on their lingual aspects which perform a similar function to the infundibula. The presence of infundibula may explain why the upper cheek teeth have less peripheral enamel folding than the lower cheek teeth. There is an intimate relationship between enamel and dentine in which dentine supports the more brittle enamel by dissipating the forces of mastication through its more resilient matrix, whilst the higher wear resistance of enamel helps to protect the softer dentine from excessive wear. A similar relationship exists between enamel and cement. The gross arrangement of dental tissues in equine teeth appears to be a compromise between the requirement for hardness and elasticity.

4.2.2 Enamel thickness

The results of the current study indicate that the cheek teeth enamel of the modern horse (*E caballus)* is five to six times thicker than the enamel of primitive

horses. The maximum values of enamel thickness of primitive (pre-Meocene) equidae recorded from fossils by Kazowa (1992) corresponds with the minimum values recorded from *E caballus* in this study. Kazowa recorded a maximum enamel thickness of 1.5 and 2 mm respectively for upper and lower molars of *E. caballus*, which is greater than the maximal values (1.2 and 1.6 mm) recorded in this study. However, the current study showed that many factors, including enamel location and numbers of measurements can significantly affect the results. As enamel thickness values are a normally distributed data, the sole use of maximum values, as used by Kazowa (1992) has limitations and the use of mean (±SD) as used in the current study is statistically more valid. Despite the apparent differences in enamel thickness between primitive and modern horses, the enamel ultrastructure of *E. caballus* is claimed to be similar to that of the ancestral equidae, even to that of the most primitive horse, *Hyracotherium* by Kazowa (1992). Thus, Kazowa claims that the enamel ultrastructure of the modern horse was present prior to the evolution of hypsodonty.

The linear enamel measurements utilised in the current study were believed to be of little value by Martin (1985) for comparing enamel thickness in hominid species (all brachyodont). Martin favoured calculating enamel thickness by dividing the total enamel volume by the surface area of the amelodentinal junction. This technique requires the calculation of total enamel volume which in turn requires three dimensional measurements of enamel, which is easier in brachyodont than hypsodont teeth, since in brachyodont teeth, enamel is distinctly separate from dentine and cement. In equine teeth however, the enamel is embedded in the other dental tissues and also has an irregular configuration which additionally varies throughout the tooth

length. These factors would make it extremely difficult to measure either the surface area of the amelodentinal junction or the total enamel volume in hypsodont teeth.

The objections raised by Martin (1985) to linear enamel measurements can be overcome by measuring enamel thickness at a large number of well defined points along the enamel folds (in the transverse plane) in all specimens. In this study, enamel thickness at fixed sites in the transverse plane was found to be relatively constant at all vertical levels of the teeth and this indicates that linear measurement of enamel thickness can be used to compare enamel thickness between horses of different ages.

This study found that on transverse sections, the thickness of enamel varies significantly at different sites along its folds. Enamel was particularly thick at the buccal (lateral) aspect of the upper and at the lingual aspect of the lower cheek teeth, which are termed the "leading sides" of the occlusal surface (Rensberger & Koenigswald 1980). The leading side represents the occlusal aspect which withstands the greatest masticatory pressure whilst grinding food, whilst the opposite side, i.e. the palatal (medial) aspect of the upper and the buccal (lateral) aspect of the lower cheek teeth are termed the "trailing sides" of masticatory movement where the enamel folds are thinner than in the leading side.

The occlusal surfaces of equine cheek teeth are not horizontal as is often the situation with brachyodont teeth, but are sloped. In the upper cheek teeth, the gingival to occlusal surface distance is longer at the buccal than at the palatal aspect and this situation is reversed in the lower cheek teeth, with a downward slope in the lingual to buccal direction. The angulation of the occlusal surface is caused both by the anatomical configuration of the maxillary and mandibular cheek teeth rows and by different rates of dental attrition. The angulation of the occlusive surface in equine

equine cheek teeth shows that attrition is higher in the palatal (medial) direction in the upper and in the buccal (lateral) direction in the lower cheek teeth. These directions correspond with the direction of the masticatory movement (medial) on the occlusal surface of equine cheek teeth.

In addition, the angulation of the occlusal surface of equine cheek teeth indicates that the thicker enamel has a lower attrition rate. This means that there is an inverse correlation between attrition rate and enamel thickness of cheek teeth. If a positive correlation existed between attrition and enamel thickness, attrition would be higher in thicker enamel and this would cause the occlusal surface of enamel to become level or even angled towards the opposite side, both of which are clinical abnormalities. The angulation of the occlusal surface of equine cheek teeth not only increases their surface area but also modifies the masticatory forces placed upon the teeth. Therefore, the regional variation in enamel thickness may not only determine how enamel resists attrition, but also may indicate how it can efficiently bear the mechanical forces of mastication.

The present study showed that equine enamel was thinner in invaginations than in ridges. Fossil evidence has shown that during evolution, the enamel of many species became thinner in certain regions of the tooth and this may have been in response to reduced masticatory forces in localised areas (Rensberger & Koenigswald 1980; Koenigswald & Clemens 1992). The hypothesis derived from these observation suggests that the evolutionary development of enamel thickness is dependant on the forces to which it is subjected during mastication. Extrapolating from this hypothesis the occlusal pressure on equine cheek teeth is lower in enamel in invaginations than enamel in ridges.

Enamel invaginations are believed to be primarily formed to increase the occlusal surface of enamel. As a result, the surface area of the amelodentinal junction at invaginations has become smaller than the area of the amelocemental junction (peripheral). The results of this study showed that the diameter of enamel prisms increases significantly towards the amelocemental junction and this increase in prism size is accompanied by a proportional decrease in the interprismatic area. During dental development in young animals, particularly after the completion of deposition of interprismatic enamel in eq. type 1 enamel, the ameloblasts may become overcrowded with a resultant decrease or cessation in enamel formation. The observed reduction in amounts of eq. type 2 enamel in the invaginations is evidence of reduced ameloblast activity.

A comparison of the enamel thickness between upper and lower cheek teeth enamel in the present study shows that regional variation in enamel thickness is greater in the upper than in the lower cheek teeth. Rensberger and Koenigswald (1980) suggested that regional variations of enamel thickness are determined by the angle of incidence of masticatory stresses to the occlusal surface. This would indicate that the distribution of masticatory stress over the occlusal surface of enamel is more complex in the upper than in the lower cheek teeth of horses.

4.2.3. Pattern of the occlusal surface

The presence of an organic pellicle over the occlusal surface of enamel of some horses but not others, may depend on the diet and management of individual horses. This organic layer may partly act as a barrier between the exposed crystals of the upper and lower cheek teeth enamel during mastication and may help reduce attrition between enamel crystals of opposing teeth. Diet can also effects the

ultrastructural appearance of the enamel surface as reported by Walker et al (1978) who examined the wear features of the occlusal surface of teeth in the browsing *Hereohyrax brucei* and the grazing *Parcavia johnstoni* and found a relationship between the ultrastructure of the occlusal surface and diet of horses. Microwear analysis of occlusal wear revealed prisms to be elevated into relief in *H. brucei* throughout the year but in *P. johnstoni* only during dry seasons with this prism relief disappearing in *P. johnstoni* during wet seasons.

The changing microwear features of *P. johnstoni* was found to be due to a shift from diet containing highly abrasive cellulose and lignin during the dry season, to a diet of vegetation containing high levels of phytoliths in wet seasons. Phytoliths cause striations which lead to the disappearance of prism relief. The observation of permanent prism relief on the occlusal surface of the browsing *H. brucei* was due to its diet, permanently high in abrasive cellulose and lignin, that expose the enamel prisms similar to that seen in the grazing *P. johnstoni* during the dry season. Rensberger and Koenigswald (1980) interpreted the above findings as evidence of differing resistance of enamel ultrastructure to wear.

At low magnification, the occlusal surface of equine enamel was found to contain numerous shallow depressions of variable sizes and shapes. The occlusal surfaces of the teeth of some hypsodont herbivorous rodents have large depressions that are caused by high masticatory forces between the upper and lower teeth and these features are regarded as a common characteristic of all hypsodont teeth by Rensberger (1978). The irregularities present on the occlusal surface of enamel are additional to the larger irregularities present over the whole occlusal surface, such as the undulations between enamel and dentine. The occlusal surface of equine enamel

also contains grooves and ridges which correspond respectively to the "wear facets" and "wear reliefs" of Fortelius (1985).

4.2.4. Incisor enamel

The enamel prisms of equine incisors have a very complex orientation, which includes the vertical interception of some prism bundles with the occlusal surface. A similar orientation of prism bundles occurs on the occlusal surface of enamel in rhinoceroses and tapiroids and are marked by the presence of ridges oriented perpendicular to the amelodentinal junction (Rensberger & Koenigswald 1980). These ridges result from a change in the direction of the horizontal prism bands of primitive mammalian enamel into the vertical alignment that is observed in more highly developed mammalian enamel (Rensberger & Koenigswald 1980). The vertically oriented prism bands occur mainly within lophs which are the ridges that connect dental cusps (Miles and Grigson 1990). Lophs represents areas of the occlusal surface where masticatory pressures are highest and consequently where attrition is greatest. Modifications in prism band orientation is thought to have evolved to extend the life of the lophs (Rensberger and Koenigswald 1980).

However, this vertically oriented enamel although highly wear resistant, is very susceptible to cracking. The current study showed that vertical prism orientation does not exist in equine cheek teeth enamel and Rensberger and Koenigswald (1980) also noted in an unspecified number of samples or sites, that this modification rarely occurs in the horse. Rensberger and Koenigswald claimed that the more oblique prism decussation present in horses is evidence that equine enamel is more primitive than the enamel of rhinoceroses or tapiroids. However, as noted by Koenigswald and Clemens (1992) and also shown in the present study, a selected characteristic can simply be a

description of a localised part rather than being representative of a whole complex structure such as a tooth. If a realistic comparison is to be made between the enamels of various species, the qualitative and quantitative ultrastructural complexities of all areas of enamel and also its relationships with other dental tissues must be taken into account.

4.2.5. Enamel ultrastructure

In order to minimise the effects of local variation in enamel ultrastructure, large numbers of teeth from different horses and large number of specimens in both the transverse and longitudinal planes from each of these teeth were examined in the current study. A combination of qualitative and quantitative analyses were then used to elucidate the structure of equine enamel at different sites within the teeth. However, quantitative examination of enamel prisms and interprismatic distances showed significant differences within individual teeth, depending on the site in the transverse plane, as well as on the vertical level of the section, thus indicating that particular enamel characteristics are not equally distributed at all sites on the tooth surface, nor at all vertical levels of the teeth. Consequently, the value of quantitative analyses of a limited numbers of characteristics to identify taxonomic relationships between various mammalian taxa, particularly the equine lineage, is questionable especially when small numbers of samples are examined.

The terminology of enamel ultrastructure is confusing and varies greatly between authors (Koenigswald and Clemens 1992). Consequently comparison of findings between workers will remain very difficult until a standardised terminology is adopted by all. However, these difficulties can be partly overcome if the identification criteria utilised in each study are clearly defined. In the current study, three enamel

types were defined both by the transverse shape of their enamel prisms and by the amounts and distribution of their interprismatic enamel. Boyde (1964) also defined three enamel types termed Boyde's type (pattern) 1, 2 and 3 mammalian enamel, according to the transverse shape of prisms and the prism packing pattern (arrangement).

Other authors, including Koenigswald and Clemens (1992) and Pfretzschner (1992) have used the term "enamel type" in hypsodont teeth to simply describe similar orientation of prisms within a unit area, regardless of the transverse shape of these prisms or of the amounts and distribution of their interprismatic enamel. Koenigswald and Clemens (1992) described four mammalian enamel types termed; radial enamel, tangential enamel, H-S bands and irregular decussation. Pfretzschner (1992) described three enamel types in hypsodont mammals, termed 3-D-enamel (three dimensionally interwoven bundles of prisms), vertical H-S bands and modified radial enamel.

In the current study, eq. type i enamel (the inner enamel layer) consisted of small prisms, ovoid shaped on transverse section which were arranged in regular columns that were separated by thick plates of interprismatic enamel. Enamel, similar to equine type 1 enamel, has been found in the inner enamel layer of other perissodactyls and also in artiodactyls (Pfretzschner 1992) and in the deeper and intermediate layers of caprine teeth (Grine et al 1987), but only as a localised patchy layer in hominoids (Martin et al. 1988). This type of enamel corresponds morphologically to Boyde's type 2 mammalian enamel (Boyde 1964). Many authors (Osborn 1981; Fortelius 1985; Grine et al 1987; Martin et al. 1988) support Boyde's classification, however others, including Gannt (1979) and Koenigswald and Clemens (1992) have reservations on its value, because they claim that Boyde's classification

uses limited characteristics, i.e. the transverse appearance of prisms and prism packing pattern, to determine enamel type and thus it fails to encompass variation within enamel types.

This criticism could also possibly be applied to the current study. However in contrast to Boyde who used her classification to compare very diverse types of enamel between many different species, it was utilised in just a single species in this study. Multiple sites in both the transverse and longitudinal planes were examined in large numbers of teeth, which should eliminate local variations in enamel types. Having classified equine enamel types into the 3 equine types, additional examinations including quantitative measurements, examination of the three dimensional orientation of enamel prisms and for the presence of H-S bands were then performed.

The resistance of enamel to wear largely depends on the angle of incidence of enamel prisms to the direction of masticatory stress (Koenigswald and Clemens 1992), with wear resistance increasing when prisms are oblique or vertical to the occlusal surface and decreasing if prisms are parallel to it (Fortelius 1985). Eq. type 1 enamel is composed of closely packed prisms which forms a composite structure with its interprismatic plates, both of which are inclined at oblique angle towards the occlusal surface. These three characteristics confer very strong wear resistance on enamel (Fortelius 1985; Koenigswald and Clemens 1992). The latter authors have also suggested that the purpose of having different enamel types in the enamel folds is to spread the applied forces and thus prevent cracks in one type of enamel from propagating across the full enamel thickness. However, the current study indicates that the marked branching present in interprismatic enamel of eq. type 1 enamel and the absence of parallel orientation of prism rows of due to the presence of prism

decussation should protect eq. type 1 enamel from cracking. Thus, the presence of more than one type of enamel may not be necessary in equine teeth for dissipating the masticatory forces that could generate major enamel cracks. Some areas of equine cheek teeth such as the caudal aspect of the mesial infundibulum consists of eq. type 1 enamel only which further supports the theory that several enamel types are not essential for crack resistance.

The transverse shape of prisms in eq. type 2 enamel (the outer enamel layer) is similar to Boyde's type 3 mammalian enamel, in that prisms are arcade or C shaped on transverse section and interprismatic enamel is absent (Boyde 1964). Enamel, similar to eq. type 2 enamel, is also found in the outer layer of caprine molars (Grine et al 1987), as a dominant pattern in human (Gantt 1979; Boyde & Martin 1982) and some non-human primate enamel (Martin et al 1988).

Ultrastructural examination of equine enamel showed that eq. type 1 enamel predominates in ridges and type 2 in invaginations and these features were also found in some fossils of primitive equidae (Fortelius 1985). In the current study, the dominance of eq. type 2 enamel in invaginations was not due to increased amounts of eq. type 2 but to a reduction of eq. type 1 enamel, with the overall enamel thickness being lower in invaginations than in ridges. Fluctuations in the thickness of both eq. types 1 and 2 enamel determines the ratio of these two types within all enamel regions in the transverse plane.

The appearance of eq. type 3 enamel on transverse section is similar to that of Boyde's type 1 mammalian enamel. Although it was identified as a separate enamel type in the present study, eq. type 3 enamel could also be considered as being the initial aspect of eq. type 1 and as the terminal aspects of eq. type 2 enamels, where the

enamel prisms taper to an end and are surrounded by an increasing amount of interprismatic material. Enamel prisms with similar transverse shape to eq. type 3 enamel are found throughout the full enamel thickness of many sirenians, whales, insectivores and bats (Fortelius 1985), adjacent to the amelodentinal junction and dental surface of hominoids (Boyde & Martin 1982; Martin et al. 1988) and some other mammals (Fortelius 1985). Enamel similar to eq. type 3 enamel is more abundant in primitive mammals than is enamel similar to eq. types 1 or 2 enamel and thus it is considered to be less highly evolved than the latter types (Fortelius 1985; Martin et al. 1988). Consequently, the presence of very small amounts of eq. type 3 enamel in horses suggests that equine enamel is highly specialised and evolved.

The present findings showed that equine enamel includes the four enamel "types" of Koenigswald and Clemens (1992) i.e. radial enamel, tangential enamel, H-S bands and irregular decussation in a combined pattern. The presence of poorly defined and irregular boundaries between different enamel types that was observed in this work has also been reported in other species (Fortelius 1985; Koenigswald and Clemens 1991). The eq. type 1 enamel described in this study is referred to as "modified radial enamel" by both Koenigswald and Clemens (1992) and Pfretzschner (1992), because it contains thick plates of interprismatic enamel and parallel columns of prisms that are obliquely inclined towards the occusal surface. Examination of equine fossils indicates that the evolutionary development of these thick interprismatic enamel plates was accompanied by an increase in crown height, i.e. hypsodonty (Kozawa 1992).

Our measurements confirmed that adjacent to the amelodentinal junction, the thickness of the interprismatic enamel plates of eq. type 1 enamel are similar to the

diameters of prisms. This indicates that interprismatic enamel which is adjacent to the amelodentinal junction must withstand the same masticatory stresses as prisms. This is a feature that differentiates eq. type 1 enamel (Pfretzschner's (1992) "modified radial enamel") from other enamel types. The current study also showed that the columns of eq. type 1 enamel prisms radiate out from the amelodentinal junction towards the amelocemental junction and that adjacent bundles of prisms decussate at various angles, forming what Koenigswald and Clemens (1992) term, an irregular prism decussation pattern, which until now has been regarded as a characteristic of Elephantoidea (Kozawa 1992).

The transverse appearances of eq. type 2 prisms remain the same even when individual prisms decussate within the enamel folds. For this reason, the enamels classified as tangential enamel and H-S bands by Koenigswald and Clemens (1992) are combined in this study. At the amelocemental junction, prisms of eq. type 2 enamel are oriented obliquely, while adjacent to their boundary with eq. type 1 enamel, eq. type 2 prisms change their orientation and become even more oblique (fig 28). As previously noted, the term diazone refers to enamel bands whose prisms are sectioned transversely and parazone, to enamel bands with vertically sectioned prisms (Boyde 1990). In vertical sections of upper cheek teeth peripheral enamel, eq. type 2 enamel shows diazone and parazone, particularly at its thickest buccolingual aspects.

As previously noted, the orientation of these bands are regarded as an important characteristic in evolutionary studies of ungulate teeth. This is because the development of different enamel types in combination with different orientation of prisms are believed to increase the resistance of enamel against the propagation of cracks and also against the attrition of masticatory forces (Rensberger 1992;

Pfretzschner 1992). Additionally, the distribution pattern of the different enamel types, particularly the development of an outer enamel layer that has decussation of its prisms rows may also be important in reducing the traumatic effects of mastication. The outer enamel layer forms the leading side of the enamel surface, where masticatory forces are greatest and where cracking is most likely to occur (Rensberger & Koenigswald 1980). This outer, decussated layer is particularly well developed in equine incisor enamel which indicates that these teeth have evolved to be highly resistant to crack propagation.

The evolution of a variety of enamel types in equine enamel has disadvantages as well as advantages. The regular columnar arrangement of prism rows in eq. type 1 enamel allows prism bundles to be tightly packed and this increases enamel hardness. This characteristic, along with the oblique inclination of prisms towards the occlusal surface increases wear resistance (Fortelius 1985; Koenigswald and Clemens 1992; Rensberger 1992). However, the presence of parallel prism rows alternating with parallel interprismatic enamel layers could readily allow cracks to propagate along the prismatic and interprismatic boundaries (Fortelius 1985; Rensberger 1992). This potential defect is overcome by the irregular decussation of prisms bundles in eq. type 2 enamel. On the other hand, the varying orientation of prisms in eq. type 2 enamel produces less closely packed bundles of prisms which in turn reduces the hardness of the layer. Consequently, the combination of various enamel types of equidae appears to be a compromise between resistance to wear and resistance to crack propagation.

The presence of thin enamel in equidae and bisons is claimed to be due to reduction or loss of the outer enamel layers, the equivalent of eq. type 2 enamel (Koenigswald and Clemens 1992) which is in complete contrast to the findings of this

study. For example, we found that the thin enamel in the mesial aspect of the caudal infundibulum contained both eq. types 1 and 2 enamel in equal thickness. In addition, the thin caudal interdental aspect of the peripheral enamel of the upper cheek teeth primarly consisted of eq. type 2, rather than eq. type 1 enamel. These findings suggests that the thinning of enamel is not necessarily due to the loss of external layer (equivalent of eq. type 2) but can also be due to the loss of internal layer (equivalent of eq. type 1 enamel) or to a proportional thinning of both types.

In this study, the prisms of all three equine enamel types possessed different shapes on transverse section and showed that the plane of section of enamel specimens has a major influence on the recorded prism shape, a feature previously noted in studies on human enamel (Eisenberg 1938; Hinrichsen & Engel 1966; Fosse 1967). Fosse (1967) studied the shapes, patterns and density of prisms in sections cut parallel and perpendicular to the incremental (growth) lines of human enamel. In sections cut perpendicular to the incremental lines, prisms were found to be vertically compressed with an almost flattened rectangular appearance and with the horizontal diameters markedly greater than vertical diameters, while sections cut planoparallel to the incremental lines showed hexagonal shaped prisms.

Examination of eq. enamel prisms suggests that during enamel formation, equine ameloblasts have four distinct periods of activities. Initially, secretory activity at the amelodentinal junction increases sharply until prisms reach circa 10 μm in length, which corresponds to eq. type 3 enamel. This is followed by a long, steady period of growth which corresponds with the secretion of eq. type 1 enamel, whose well-organised prism orientation suggests that ameloblasts follow very regular courses and during this second period, prism sizes remain constant. In the third period of growth

(eq. type 2 enamel), ameloblastic secretion sharply increases to its highest level and continues so until the amelocemental junctions is reached. During this third growth period, two types of complexity occur in relation to prism orientation. The first type is random and is limited to differences in direction between individual prisms, but the second type of complexity, which is believed to be genetically determined (Boyde 1978), involves differences in direction between bundles of prisms, as is manifested by the presence of decussation and H-S bands.

In the fourth and final period of enamel growth there is a sharp reduction in ameloblastic secretion. This decrease of prism sizes at the amelocemental junction and the aforementioned sharp increase of prism sizes at the amelodentinal junction causes prisms to have pointed shapes at these locations. It is perhaps these growth characteristics that produce eq. type 3 enamel at both the amelodentinal and amelocemental junctions.

Both polarised light and SEM examinations showed that regular diazone and parazone were only present in certain areas of eq. type 2 enamel, particularly in the thickest buccal aspects of upper cheek teeth enamel, rather than being a constant characteristic of all eq. type 2 enamel. In an unrecorded numbers of specimens and without giving any detail of their distribution within the teeth, Kozawa (1992) also recorded the presence of regular diazone and parazone in the outer layer of equine enamel and considered this to be an ungulate characteristic.

The quantitative analyses in this study showed eq. type 1 prisms to be significantly smaller than eq. type 2 prisms. In other mammals, enamel prisms similar to eq. type 1, were also found to be smaller than those corresponding to eq. type 2 enamel prisms (Boyde 1969). These observations show that differences between

enamel types are not limited to the shapes of prisms in transverse plane, the amounts of interprismatic enamel (Boyde 1964) or the three dimensional orientation of individual and bundles of prisms (Koenigswald and Clemens 1992). Prism sizes and the rate of secretion of prisms by ameloblasts, as determined by the intervals between successive incremental lines are also considered to be valuable features for enamel classification (Martin 1985). Secretion rate, prism sizes, homogeneity of enamel ultrastructure and enamel thickness are interrelated and by utilising these criteria hominid enamel has even been divided into 7 types by Martin (1985).

The current study shows the presence of an inverse relationship between prism diameter and interprismatic distance, with prism diameters increasing where interprismatic distance decreased, i.e. towards the amelocemental junction (peripherally). However, these changes were not proportional, with the increase in prism diameter far greater than the decrease in interprismatic distance. Consequently, as the numbers of prisms remain constant between the amelodentinal and amelocemental junctions (Fortelius 1985), the disproportional increase of prism diameter of equine cheek teeth is accommodated by the larger enamel surfaces of the amelocemental junction.

The current study showed the presence of several ultrastructural differences between maxillary and mandibular cheek teeth enamel. Greater amounts of both eq. types 1 and 2 enamel were present in maxillary than mandibular cheek teeth, with eq. type 1 dominating maxillary and eq. type 2 dominating mandibular cheek teeth enamel. As previously noted, eq. type 1 enamel has higher wear but lower crack resistance than eq. type 2 enamel and the converse is also true. Thus, the ultrastructure of maxillary cheek teeth enamel may have evolved to allow this enamel

to be more resistant to attrition, whilst mandibular cheek teeth enamel is more resistant to crack propagation. These ultrastructural variations may indicate that equine maxillary and mandibular cheek teeth are subjected to different masticatory forces. The other major difference between the enamel of these teeth is in the distribution of enamel types along the folds. Mandibular cheek teeth contain both types along all its enamel folds. In contrast, some localised areas of maxillary cheek teeth enamel contain just one of these types. As eq. types 1 and 2 enamel shows a compromise between wear resistance and hardness, mandibular cheek teeth enamel appears to accommodate both of these characteristics better than maxillary cheek teeth.

Comparison between cheek teeth and incisor enamel also shows several significant differences. The prism diameters of eq. type 1 enamel were greater in incisor than in cheek teeth enamel. However, the main differences between these two types of teeth was that much less eq. type 1 enamel is present in incisors than in cheek teeth. Additionally, the orientation of prism rows within all 3 individual types of enamel was more complex in incisor than in cheek teeth enamel. For example, diazone and parazone were only seen in the peripheral enamel of the maxillary cheek teeth but were present throughout incisor enamel, often extending for almost the full enamel thickness.

On transverse sections of maxillary cheek teeth, the orientation of eq. type 2 enamel prisms ranged from oblique angles to being nearly horizontal to the occlusal surface, whereas in incisor enamel they were oriented in multiple directions, including in oblique, vertical and horizontal planes. This multi-directional prism decussation in incisors should have significant influence on crack propagation by dissipating

masticatory forces. When the masticatory function of incisors and cheek teeth enamel are compared, the reason for these variations become apparent. Equine incisors are much smaller and flatter, and also have less support from adjacent teeth than occurs with cheek teeth. They function in prehension and cutting and thus undergo great compressive forces that could readily cause cracks. Additionally, when horses graze they may bite solid structures like small stones and so a high crack resistance is essential in incisor enamel and its very highly decussated eq. type 2 enamel content fulfils this requirement. Cheek teeth primarily have a grinding function and so the presence of enamel with higher wear resistant is more essential in cheek teeth enamel and in turn, this requirement is fulfilled by the high eq. type 1 enamel content of cheek teeth.

In conclusion, the qualitative and quantitative findings of this study suggest that the ultrastructure of equine enamel are related to both the gross shape of teeth and also to their function. Consequently, there are greater ultrastructural differences between cheek teeth and incisor enamel than between upper and lower cheek teeth enamel.

CHAPTER 5

GROSS, LIGHT MICROSCOPIC AND ULTRASTRUCTURAL EXAMINATIONS OF DENTINE

RESULTS

5.1. Light microscopy of dentine

5.1.1. Orientation of dentinal tubules

In vertical (longitudinal) sections of teeth, the dentinal tubules were observed to extend from the amelodentinal junction to the pulp cavity in various orientations, depending on their sites in the teeth. These sites and orientations are summarised in **table 10**.

UCT	LCT	Dentinal sites	Orientation of dentinal tubules
+	+	Cusp tips	Straight and vertical
+	+	Flanks (sides) of crown	Oblique: Degree of angulation decrease towards the tooth apices
+		Apices of infundibula	S shaped curvatures: Adjacent tubules form a line parallel to the amelodentinal junction
+		Parietal infundibular	Oblique following a small curves at the ADJ: Curved adjacent tubules form a line parallel to the ADJ
+		Inter-infundibular	Large oblique curves

Table 10: Orientation of the dentinal tubules in vertical sections of upper cheek teeth (UCT) and lower cheek teeth (L CT)

The dentinal tubules in cusp tip dentine followed a straight and vertical course towards the pulp (fig 44). Peripheral dentinal tubules were oriented at oblique angles to the pulp cavities (fig 45) with the degree of angulation decreasing from crown to apex. Dentinal tubules originating at the apices of the infundibula ran towards the pulp cavities with an S shaped curvature (fig 46). The tubules of infundibular parietal (wall) dentine initially formed small curves near the amelodentinal junction and then proceeded at oblique angles toward the pulp cavities (fig 47). Additionally at this site, rows of curved adjacent tubules formed a series of lines parallel to the amelodentinal

junction (fig 47). The tubules of dentine between the two infundibula (inter-infundibular dentine) extended toward the pulp cavity forming large oblique curvatures (fig 48).

On transverse sections of teeth, the dentinal tubules were sectioned in various planes depending on their sites within the teeth. The dentinal tubules surrounding large pulp cavities were found to be sectioned in the transverse plane and on ground sections, these tubules appeared as small, round structures located at the centre of the white, rounded area of peritubular dentine (fig 49). Equine cheek teeth have gross ridges and furrows on their occlusal surface that are particularly evident when a tooth is viewed from the buccal or lingual aspects (fig 4). In transverse sections of these ridges, dentinal tubules were found to be sectioned in various planes including transversely, obliquely and longitudinally, depending on their position within the ridge (fig 50). Dentinal tubules originating at the tips of ridge dentine were sectioned either obliquely or longitudinally and followed straight courses towards the pulp cavities (fig 50). After a short vertical orientation close to the amelodentinal junction, the tubules from the flanks (sides) of ridge dentine were found to bend at various angles toward the pulp cavities (fig 50). The plane of section in relation to location of dentinal tubules are summarised in **table 11**.

UCT	LCT	Dentinal sites	Plane of section of the dentinal tubules	Location of dentinal tubules within peritubular dentine
+	+	Surrounding large pulp cavities	Transverse	Peripheral
+	+	Beneath the dental cusps	Transverse	Central
+	+	Ridge-tips (apices)	Longitudinal	-
+	+	Ridge-flanks (sides)	Oblique and transverse	Variable in transversely sectioned peritubular dentine
+	-	Interinfundibular	Oblique and transverse	Variable in transversely sectioned peritubular dentine

Table 11: Location of dentinal tubules within peritubular dentine of primary dentine in transverse sections of upper cheek teeth (UCT) and lower cheek teeth (LCT).

5.1.2. Contents of dentinal tubules

Light microscopic examination of ground dentinal sections showed the lumina of dentinal tubules to be filled with structures that resembled odontoblast processes and these structures usually extended uninterruptedly throughout the full lengths of the dentinal tubules (figs 44, 46, 47 & 48). On LM examinations of decalcified and stained sections, similar structures were observed, but unlike in ground sections, these structures did not completely fill the tubular lumina and also were usually discontinuous throughout the tubule length (fig 50).

LM examinations of ground and stained sections showed most dentinal tubules to divide into two or three branches adjacent to the amelodentinal junction (figs 46, 47, 48, 49 & 51). LM examination of ground sections also revealed that some of these branches actually extended into the enamel (figs 46, 48 & 49). Intertubular dentine appeared as dark brown or pink areas in decalcified sections stained with H & E and as dark grey areas in ground sections. In transverse sections of equine cheek teeth, dentine contained several distinct regions between the amelodentinal junction and the pulp cavities (figs 51 & 52). The sizes and numbers of dentinal tubules, location of tubules in peritubular dentine, diameter of peritubular dentine and the ratio of areas of peritubular dentine: intertubular dentine differed for each particular dentinal region.

The amount of intertubular dentine decreased from the amelodentinal junction towards the junction of primary and secondary dentine (figs 51 & 52), whilst the diameter and area of peritubular dentine increased in this direction (figs 51 & 52). However, at the junction of primary and secondary dentine, the diameter and area of peritubular dentine decreased sharply, but the amount of intertubular dentine and the

number of dentinal tubules increased (fig 52). High power LM examinations revealed a transitional zone between primary and secondary dentine (fig 52). Peritubular dentine was absent in regular secondary dentine, but the amounts of intertubular dentine and the density of dentinal tubules/unit area was higher in secondary than in primary dentine (fig 53).

5.1.3. Incremental (growth) lines

LM examinations of decalcified dentinal specimens showed two types of incremental lines in primary equine dentine, one thin and one thick. The thin incremental lines were inconsistently present in transverse sections of teeth and ran parallel to each other, displaying minute curves along their courses (fig 54). These lines were found to correspond with constrictions and dilatations of adjacent tubules and were arranged vertical to the long axes of tubules. These thin incremental lines appeared as dark brown lines with grey interincremental areas. Data pertaining to the distances between these thin incremental lines were normally distributed and these data are presented as mean (±SD). The mean (±SD) distance between these incremental lines was 34.49 ± 9.42 μm in the upper and 29.67 ± 7.16 μm in the lower cheek teeth.

Thick incremental lines were also detectable on low magnification LM examination. These lines also had a dark brown appearance and were oriented almost planoparallel to the amelodentinal junction (fig 55). In ground sections, thick incremental lines were only observed in dentine surrounding the apical aspects of infundibula (upper cheek teeth) (fig 46). In transverse sections of dentine, the intervals between adjacent thick incremental lines were irregular and data relating to distances between the thick incremental lines were non normally distributed. Therefore, the

combined values of these measurement in both upper and lower teeth are given as median (51.52 μm) and range (22.16 to 183.68 μm). These intervals were usually greatest in ridge dentine and lowest in invagination dentine. Although major differences occurred in the intervals between successive thick incremental lines, the incremental lines themselves were relatively uniform in thickness (mean 13.58 ± 4.91 μm). The pattern of these lines, which were also visible in decalcified sections in the SEM, were unique to particular teeth.

5.1.4. Predentine

LM examination of decalcified and H & E stained dentinal sections revealed a thin, pale band, termed predentine to be intermittently present between the mineralised dentine and the pulp (fig 56). The thickness of this band varied along the pulpodentinal interface and it was absent in some areas (fig 57).

5.2. Scanning electron microscopy of dentine

5.2.1. Dentinal tubules

On high power SEM examinations, the dentinal tubules were observed to terminate close to the amelodentinal junction and therefore an irregular, tubule-free region was always identified between the distal aspects of the dentinal tubules and the amelodentinal junction (figs 58 & 59). The thickness of this tubule-free region ranged from 4.75 to 86.34 μm (median 14.66 μm). The dentinal tubules adjacent to this band had irregular sizes and appearances (fig 58).

The dentinal tubules of primary dentine were totally surrounded by a variable thickness of peritubular dentine in which they were embedded. The location of dentinal tubules within peritubular dentine varied greatly and was largely dependant on the orientation of the particular dentinal tubules **(table 11).** Where dentinal tubules

followed an oblique, straight course toward the pulp cavities, the tubules were non-centrally located in the peritubular dentine (figs 60 & 61) whilst adjacent to the pulp cavities, the dentinal tubules were located peripherally (fig 60 & 61). Adjacent to the junction of primary and secondary dentine, at which point the diameter of peritubular dentine was widest, the dentinal tubules were non-centrally located in peritubular dentine (fig 61). The dentinal tubules in ridge and invagination dentine of both upper and lower teeth appeared to be located at random in peritubular dentine (fig 62). Dentine surrounding the apices of upper cheek teeth infundibula and the dentine of both upper and lower teeth cusps contained tubules which were located centrally in peritubular dentine (figs 63 & 64).

At low power SEM magnification (x442), the dentinal tubules followed wavy courses (fig 65), but this feature was not apparent at high power magnification, where a smaller area of the dentinal tubule was visible (fig 66). In acid etched dentinal sections, where the peritubular dentine had largely been dissolved, the lumina of longitudinally sectioned tubules showed variations in thickness throughout their lengths (fig 67) with constrictions aligning with dilatations of adjacent tubules (fig 67).

5.2.2. Odontoblast processes

On SEM examination of transverse sections of decalcified dentine, the odontoblast processes appeared as thick membranous structures that completely filled the lumina of the dentinal tubules (figs 61, 63 & 64). These odontoblast processes were surrounded by an interwoven pattern of collagenous fibres that originated in the peritubular dentine (fig 64). In longitudinal sections of dentinal tubules of untreated specimens (fig 68) fibrillar attachments were observed between the odontoblast

processes and fibres of the peritubular dentine. Although most dentinal tubules contained one odontoblast process, tubules with two odontoblast processes were occasionally found (fig 69).

The dentinal tubules at the occlusal surface of most cheek teeth contained odontoblast processes (figs 70, 71 & 72) and the remarkably undamaged morphology of these structure that are continuously exposed to the oral environment suggests that they were calcified. On the occlusal surface odontoblast processes were scattered widely in primary dentine (fig 72) and the number of dentinal tubules containing odontoblast processes increased gradually from primary dentine towards regular secondary dentine with almost all dentinal tubules of regular secondary dentine containing processes (figs 70, 71 & 72). On the occlusal surface the number of dentinal tubules/unit area was significantly higher in regular secondary dentine than in primary dentine and consequently, high numbers of odontoblast processes were always observed in the mid region of regular secondary occlusal dentine (fig 71 & 72). The deposition of regular secondary dentine was followed by the deposition of irregular secondary dentine which contained no odontoblast processes and in which almost all dentinal tubule lumina were obliterated (sclerosed) (fig 70). The numbers of odontoblast processes within the dentinal tubules within the main body of the tooth differed greatly from the findings on the occlusal surface as discussed in sections 5.4, 5.5 and 5.6.

5.2.3. Peritubular dentine

Peritubular dentine was observed around the dentinal tubules of primary dentine in developing tooth germs. In contrast, the secondary dentine of permanent teeth contained little or no peritubular dentine (figs 73 & 74). Where peritubular

dentine was absent, the dentinal tubules were just surrounded by intertubular dentine (figs 73 & 74). Examination of fractured and untreated specimens of primary dentine showed peritubular dentine to have a compact appearance and a smooth surface (figs 65 & 66). Collagenous fibres were not observed in untreated sections of peritubular dentine, except for some calcified fibres which crossed the junction of peritubular and intertubular dentine (fig 68).

Acid etched sections showed peritubular dentine to have a very rough appearance and to contain a number of canaliculae, often oriented obliquely to the long axes of the dentinal tubules (fig 62). During acid etching of dentinal specimens, peritubular dentine became more deeply etched than intertubular dentine and consequently these areas were readily identified when sectioned transversely (fig 61). The mineralised components of peritubular dentine were completely dissolved during decalcification. Therefore, in decalcified sections, the sites of peritubular dentine just contained an interwoven pattern of collagenous fibres which was partially or even totally destroyed in some sections (fig 63 & 64).

Both SEM and LM examinations of primary dentine showed that the diameter of peritubular dentine increased and the amount of intertubular dentine decreased from the amelodentinal junction (figs 58 & 59) towards secondary dentine (figs 61, 62 & 64). In acid etched sections, primary dentine had a honeycomb appearance, particularly near the junction of primary and secondary dentine, where the diameter of peritubular dentine was greatest (fig 61).

On transverse sections of cheek teeth, primary dentine was found to be thicker in areas adjacent to large than to small pulp cavities. SEM examination showed a transitional region between primary and secondary dentine where peritubular dentine

was absent. In this area, the number of dentinal tubules/unit area increased and the amount of intertubular dentine was greater than in the adjacent primary dentine (figs 64, 70, 73 & 74) resulting in the disappearance of the honeycomb pattern of etched primary dentine.

5.2.4. Intertubular dentine

Intertubular dentine was found to surround peritubular dentine in primary dentine (figs 62 & 64) and the dentinal tubules in secondary dentine (figs 73 & 74). Examination of fractured untreated specimens showed that the surface of intertubular dentine was irregular and contained both calcified fibres and areas of focal mineralisation (fig 65). In untreated specimens, intertubular dentine was difficult to differentiate from peritubular dentine (fig 62). However, following acid etching or decalcification, intertubular dentine remained largely intact and was elevated above the adjacent more deeply etched or decalcified dentinal tissues (figs 58, 61, 62, 63 & 64) and consequently was readily distinguishable. The area of primary dentine containing the least amount of intertubular dentine contained the maximum amount of peritubular dentine (fig 61, 62 & 64). As the quantity of intertubular dentine decreased, its irregular, open texture was replaced by a smoother, more compact appearance (fig 61, 62 & 64).

5.3. Transmission electron microscopy of dentine

Transmission electron microscopy, like SEM examinations, showed dentinal tubules to be located at various locations within peritubular dentine (figs 75 & 76). Dentinal tubules were observed to give off minute lateral branches into the peritubular dentine and these branches contained odontoblast processes (fig 75). Most dentinal tubules were demarcated from peritubular dentine by a thin lining membrane, termed

the lamina limitant by (Torneck 1994) (figs 75 & 76). Peritubular dentine was completely lost during decalcification and in decalcified sections, its former sites appeared as empty spaces in intertubular dentine, the latter being minimally affected by the decalcification process (figs 75 & 76). Intertubular dentine contained densely packed collagenous fibres that were oriented parallel to the long axes of the dentinal tubules (fig 77). These fibres appeared to be attached to each other by the ground substance of intertubular dentine. In decalcified sections some collagenous fibrils were surrounded by empty spaces of variable thickness (fig 77).

5.4. Quantitative analyses of odontoblast processes

The density of odontoblast processes/unit area in an upper and lower M1 of 8 different horses was enumerated in three dentinal regions, (1) near the amelodentinal junction, (2) near the junction of primary and secondary dentine and (3) in regular secondary dentine and at three vertical levels (1-3; from crown to apex). The results of these measurements are presented in **tables 12 & 13**, respectively and individual values of the 8 horses are presented in appendix 13. Data were non normally distributed and were analysed using non-parametric statistical techniques.

Regions	Upper M1			Lower M1			Upper and Lower M1		
	Median	Min.	Max.	Median	Min.	Max.	Median	Min.	Max.
1	11.5	2	26	10.50	0	18	11	0	26
2	14	0	37	13	0	37	13.5	0	37
3	2	0	52	0	0	37	1	0	52

Table 12: Numbers of odontoblast processes/2202.5 μm^2 in three regions (1; in primary dentine adjacent to the amelodentinal junction, 2; in primary dentine close to the junction of primary and secondary dentine and 3; in regular secondary dentine) of upper and lower M1 of 8 horses

Statistical analysis of the median data of the 8 horses presented in **table 12** showed no significant ($p= 0.0088$) change in odontoblast process numbers between regions 1 and 2, but a highly significant decrease ($p= 0.000$) between regions 2 and 3.

Levels	Upper M1			Lower M1			Upper and Lower M1		
	Median	Min.	Max.	Median	Min.	Max.	Median	Min.	Max.
1	9	0	47	11.5	0	37	12	0	47
2	12.5	0	52	11	0	27	12	0	52
3	7	6.5	30	6	0	37	5.5	0	0

Table 13: Number of odontoblast processes/2202.5 μm^2 (combined values for 3 regions) in three vertical levels of upper and lower M1 of 8 horses

Statistical analysis of the median data of the 8 horses presented in **table 13** showed that the number of odontoblast processes did not differ significantly between vertical levels 1 and 2 of upper (p= 0.3802) or between levels 2 and 3 in both upper (p= 0.2362) or lower cheek teeth (p=0.4560).

Analysis of variance of this variable between horses and between upper and lower teeth of the same horses are presented in **table 14** which showed no significant difference in these data.

Variables		Median	Min.	Max.	Statistics	Comments
Horses	8	11	0	52	H=3.75	Non-significant
	9	12	0	47	d.f.=7	
	10	13	0	30	P=0.807	
	11	9	0	23		
	12	14	0	37		
	14	13	0	35		
	15	9	0	28		
	16	7.5	0	25		
Teeth	Upper	11	0	52	W= 4867.5	Non-significant
	Lower	10	0	37	P= 0.4499	

Table 14: Statistical analysis of number of odontoblast processes (median and range)/2202.5 μm^2 in upper and lower M1of 8 different horses

5.5. Quantitative analyses of dentinal tubules

The density of dentinal tubules/unit area was counted at the three regions that were previously used for enumerating the odontoblast processes. The results from these measurements showed that the number of tubules increased from region 1 to region 3, and that this increase was particularly marked between regions 2 & 3 (fig 78).

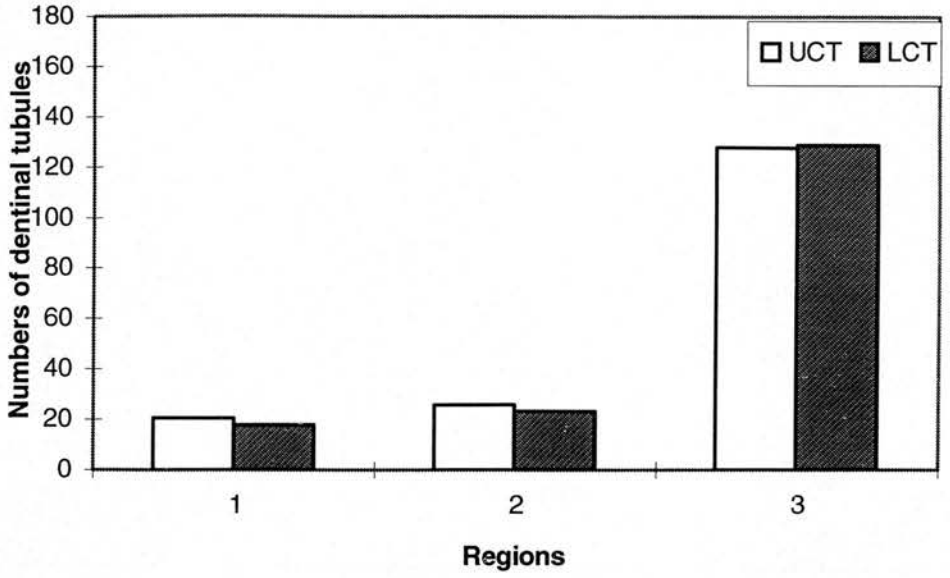

Fig 78: Median number of dentinal tubules /2202.5 μm^2 in three regions (1; in primary dentine adjacent to the amelodentinal junction, 2 in primary dentine close to the junction of primary and secondary dentine and 3; in regular secondary dentine) of upper cheek teeth (UCT) and lower cheek teeth (LCT).

This figure also shows that values between the same regions of upper and lower cheek teeth are similar. Values for individual horses are given in appendix 14. Data on numbers of dentinal tubules/unit area at three levels of upper and lower cheek teeth are presented in **table 15**.

Levels	Upper M1			Lower M1			Upper and Lower M1		
	Median	Min.	Max.	Median	Min.	Max.	Median	Min.	Max.
1	30	11	153	27	10	250	30	10	250
2	28.5	10	195	29	12	217	28.5	10	217
3	24	7	176	28	8	230	26	7	230

Table 15: Combined values of numbers of dentinal tubules/2202.5 μm^2 in three levels of upper and lower M1 of 8 horses

When these data were analysed, no significant differences were observed in the numbers of dentinal tubules between upper and lower cheek teeth, different levels or horses, but significant differences were found between the 3 different regions (**table 16**).

Variables		Median	Min	Max.	Statistics	Comments
Horses	8	28	15	154	H=2.94	Non-significant
	9	33.5	12	176	d.f.=7	
	10	38	14	127	P=0.890	
	11	30	17	195		
	12	21	14	320		
	14	29	18	250		
	15	25	9	173		
	16	30	7	150		
Levels	1	30	10	250	H= 0.29	Non-significant
	2	28.5	10	217	d.f.= 2	
	3	26	37	230	p= 0.865	
Teeth	Upper	28.5	7	195	W= 4787.0	Non-significant
	Lower	28	8	250	P= 0.6840	
Regions	1	19	7	60	H= 96.80	highly significant
	2	25	15	63	d.f.= 2	
	3	128	83	250	P= 0.000	

Table 16: Statistical analysis of number of dentinal tubules (median and range)/2202.5 μm^2 in different horses, vertical levels, teeth and regions.

5.6. Quantitative analyses of odontoblasts and dentinal tubules

The numbers of odontoblast processes and dentinal tubules/unit area was compared in the three previously described regions of dentine and the results are presented in fig 79. This figure shows that less odontoblast processes than dentinal tubules are present in all regions, but that these differences are much greater in region 3 than in region 1 or 2 with about half of the dentinal tubules in regions 1 & 2, and circa 99% of the dentinal tubules in region 3 containing no odontoblast processes.

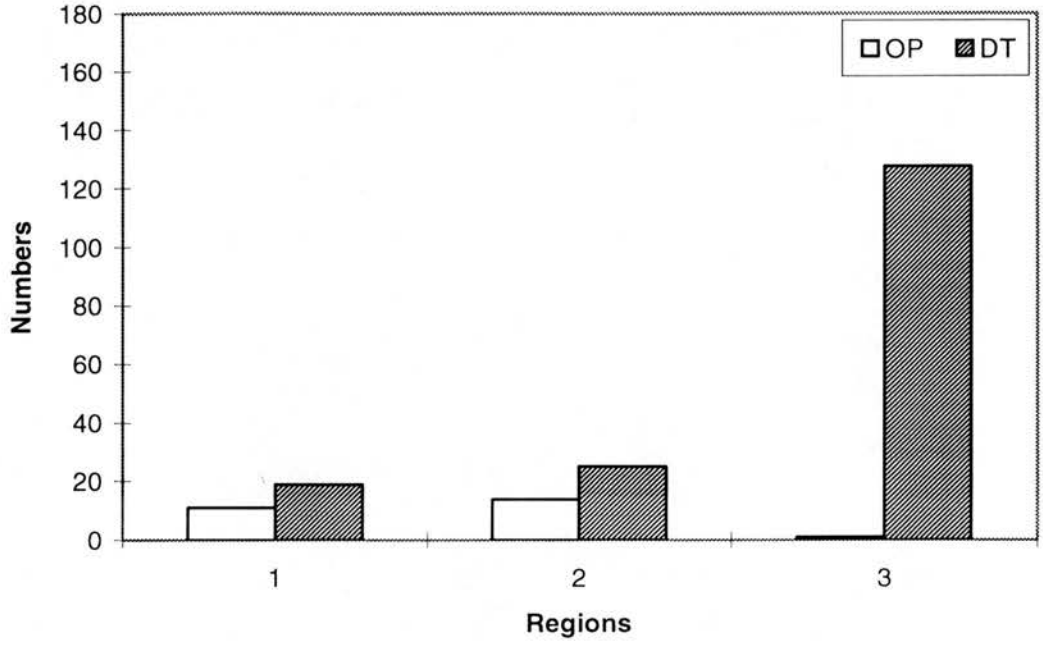

Fig 79: Median number of odontoblast processes (OP) and dentinal tubules (DT) /2202.5 μm^2 in three regions (1; in primary dentine adjacent to the amelodentinal junction, 2 in primary dentine close to the junction of primary and secondary dentine and 3; in regular secondary dentine) in combined upper and lower cheek teeth values of 8 horses.

The ratios of odontoblast processes:dentinal tubules in levels 1, 2 & 3 was respectively 8.0:30, 12.8:28.5 and 7.0:24 in upper, and 11.5:27, 11.0:29 and 6.0:28 in lower cheek teeth. No significant differences were present in this variable between the three levels of upper or lower cheek teeth.

5.7. Quantitative analyses of dentinal tubule diameter

The diameter of dentinal tubules was measured at three regions (adjacent to the amelodentinal junction, close to the junction of primary and secondary dentine and in regular secondary dentine). Mean dentinal tubule diameters in these three regions of upper and lower cheek teeth dentine are presented in fig 80 which shows that tubular diameter increases from the amelodentinal junction to regular secondary dentine.

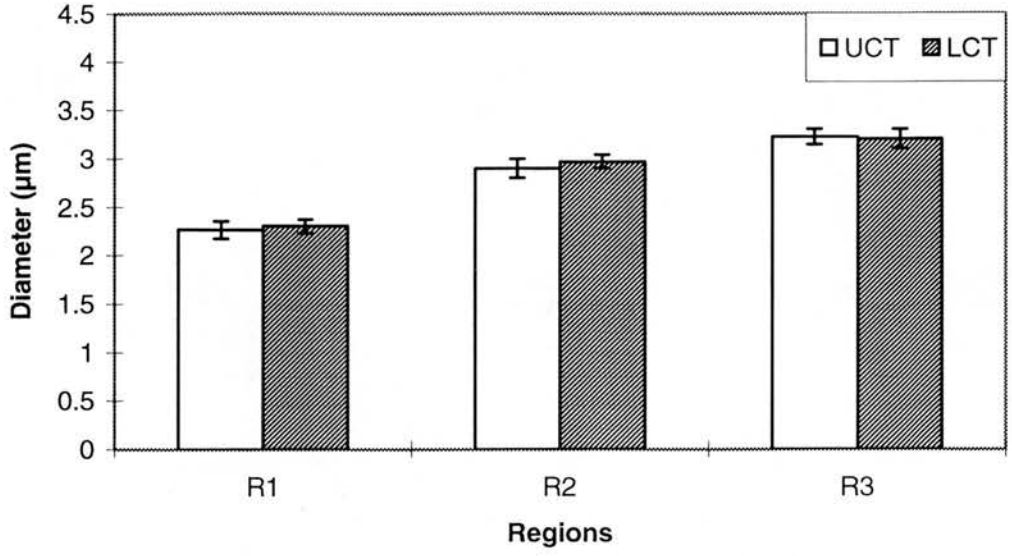

Fig 80: Diameter (µm) of dentinal tubules in three regions (R) (1; in primary dentine adjacent to the amelodentinal junction, 2 in primary dentine close to the junction of primary and secondary dentine and 3; in regular secondary dentine) of the upper cheek teeth (UCT) and lower cheek teeth (LCT).

Mean diameters of the dentinal tubules in levels 1, 2 and 3 of upper and lower M1 of the 8 individual horses are presented in appendixes 15 & 16 and analysis of variance on these data shows a highly significant difference in the diameter of dentinal tubule between the 3 different regions, but no significant difference is present between the different levels, nor between different horses or between upper and lower teeth of the same horses (**table 17**).

Source of variation	Degree of freedom	Sum square	Mean square	F Ratio	Probability
Horses	7	2.756	0.394	1.2	0.31
Error	132	43.484	0.329		
Region	2	21.300	10.650	58.50	<0.000
Error	137	24.940	0.182		
Level	2	0.024	0.012	0.04	0.965
Error	137	46.216	0.337		
Teeth	1	0.020	0.020	0.06	0.806
Error	138	46.219	0.335		
Total	139	46.240			

Table 17: Analysis of variance on diameters (µm) of dentinal tubules in upper and lower M1 of 8 horses between different horses, regions and between upper and lower teeth of the same horses

5.8. Quantitative analyses of peritubular dentine

5.8.1 Diameter of peritubular dentine

The combined values of diameters (Mean ± SD) of peritubular dentine were 8.19 ± 1.23 μm and 10.15 ± 0.62 μm respectively in regions 1 and 2 (peritubular dentine was not present in region 3, i.e. regular secondary dentine of upper cheek teeth dentine) and 8.48 ± 1.35 μm and 10.18 ± 1.87 μm in the equivalent regions of the lower cheek teeth. The combined values of the diameters of peritubular dentine at different levels of the teeth are presented in **table 18**. Data of diameter of peritubular dentine in different regions and levels of 8 individual horses are also given in appendix 17.

Levels	Upper M1		Lower M1		Upper and lower M1	
	Mean	SD	Mean	SD	Mean	SD
1	8.93	1.93	9.23	1.77	9.093	1.89
2	9.32	1.75	9.35	1.84	9.33	1.79
3	9.16	1.95	9.36	1.90	9.27	1.93

Table 18: Mean and standard deviation (SD) of diameter (μm) of peritubular dentine at three levels of upper and lower M1 of 8 horses

Analysis of variance on diameters of the peritubular dentine showed highly significant ($p < 0.001$) differences for both variables between regions 1 and 2 in both upper and lower cheek teeth (**table 19**) showing that peritubular dentine significantly increases from the amelodentinal junction towards the junction of primary and secondary dentine. However, no significant differences existed in diameter of peritubular dentine between different vertical levels of teeth (**table 19**).

Source of variation	Degree of freedom	Sum square	Mean square	F Ratio	Probability
Level (L)	2	307.9	154	1.24	0.296
Region	1	16377.3	16377.3	131.71	<0.001
Level x region	2	242.9	121.4	0.98	0.382
Error	70	8704	124.3		
Total	95	29018.0			

Table 19: Analysis of variance on diameter (μm) of peritubular dentine of upper and lower M1of 8 horses

5.8.2. Area of peritubular dentine

Mean areas (± SD) of peritubular dentine at two regions and three vertical levels of upper and lower cheek teeth of 8 individual horses are presented in appendix 18 and combined values of this variable at different regions and levels are given in **tables 20 & 21**, respectively. Analyses of variance on these data showed that area of peritubular dentine increased significantly between regions 1 & 2, but remained similar in the different vertical levels of both upper and lower cheek teeth and between different horses (table 22).

Regions	Upper M1		Lower M1		Upper and lower M1	
	Mean	SD	Mean	SD	Mean	SD
1	53.84	15.93	57.67	18.51	55.65	17.29
2	83.75	35.22	84.04	31.04	83.89	33.25

Table 20: Area (μm^2) [Mean and standard deviation (SD)] of peritubular dentine in two regions (1; near the amelodentinal junction, 2; near the junction of primary and secondary dentine) of upper and lower M1 of 8 horses

Levels	Upper M1		Lower M1		Upper and lower M1	
	Mean	SD	Mean	SD	Mean	SD
1	65.47	28.76	69.27	26.05	67.19	27.61
2	70.35	27.26	71.05	29.18	70.69	28.19
3	68.89	35.9	71.29	30.37	70.07	33.29

Table 21: Mean and standard deviation (SD) of area (μm^2) of peritubular dentine at three vertical levels of upper and lower M1 of 8 horses

Source of variation	Degree of freedom	Sum square	Mean square	F Ratio	Probability
Horses	7	13.00	1.86	1.57	0.155
Error	87	102.95	1.18		
Region	1	80.6896	80.6896	306.20	<0.001
Error	93	35.778	0.385		
Level	2	1.24	0.62	0.50	0.609
Error	92	114.71	1.25		
Teeth	1	0.74	0.74	0.60	0.441
Error	93	115.21	1.24		
Total	94	115.95			

Table 22: Analysis of variance on **area** (μm^2) of peritubular dentine in upper and lower M1 of 8 horses at different regions and levels

5.10. Quantitative analyses of intertubular dentine

The area of intertubular dentine was calculated by subtracting the area of peritubular dentine from the total dentinal area. The ratio of peritubular dentine:intertubular dentine in regions 1 and 2 were respectively, 1.45 and 2.82 in upper and 0.94 and 2.09 in lower cheek teeth. These results show that the amount of intertubular dentine decreases between regions 1 and 2. The ratio of peritubular dentine: intertubular dentine for individual horses is shown in appendix 19.

5.2. DISCUSSION

The different techniques used to examine dentine in this study, have shown different findings in respect to the dentinal tubule contents. Light microscopy of undecalcified, ground sections showed the lumina of the dentinal tubules to be completely filled throughout their lengths with odontoblast processes. These processes extended through both primary and secondary dentine from the pulp to the amelodentinal junction. In contrast, SEM examinations showed that the dentinal tubules in secondary dentine seldom contained odontoblast processes. The most likely explanation for this discrepancy is that sample preparation for SEM examination creates more artefacts than sample preparation for light microscopy and more so in soft than in calcified tissues. Soft tissues lose over a third of their volume during the "critical point drying" utilised in this study for preparation of samples for SEM examination, inevitably causing the odontoblast processes to shrink and this shrinkage could explain the absence of these processes in secondary dentine.

However, the above discrepancy is probably more complex than can be explained by shrinkage alone. In primary dentine, odontoblast processes have many side branches extending into lateral canaliculae which should help resist contraction. Additionally, within primary dentine the collagenous fibres of peritubular dentine are intimately connected to the membrane of the odontoblast processes, binding them firmly to the dentinal tubule walls and this also should prevent them from contracting. In contrast to primary dentine, equine secondary dentine contains virtually no peritubular dentine and therefore no interconnecting fibres are present between the

odontoblast processes and the dentinal tubular walls. Consequently, iatrogenic shrinkage and rupture of odontoblast processes during sample preparation should affect secondary dentine more than primary dentine. In conclusion, the finding of odontoblast processes adjacent to the amelodentinal junction in LM examination shows that the odontoblast processes in equine dentine extend as far as the amelodentinal junction.

There are also disagreements in relation to the contents of dentinal tubules in mineralised dentine in other species. SEM studies in human (Lester & Boyde 1968; Thomas 1979), rat (Jessen 1967) and feline (Holland 1975, 1976) dentine have shown odontoblast processes to be confined to the innermost (medial) layer of dentine. However, examination of dentine prepared by cryofixation have shown odontoblast processes extending as far the amelodentinal junction in monkey (Kelly et al 1981) and human dentine (Torneck 1994). These cryofixation results are supported by immunocytochemical studies (Sigal et al 1984; Sigal et al 1985) where labelled antibodies, specific for certain odontoblastic intracellular contents such as actin, vimentin and tubulin were detected within the dentinal tubules of human and rat dentine as far as the amelodentinal junction. Particularly as a result of these recent findings, it is now generally accepted that human odontoblast processes extend from the pulp to the amelodentinal junction.

Differences were also encountered between LM and SEM findings in relation to the presence of odontoblast processes at the amelodentinal junction. In LM examinations of ground dentinal sections, odontoblast processes appeared to cross the amelodentinal junction into the enamel (fig 45). The reason why some odontoblast processes appeared to cross the amelodentinal junction while other processes did not,

may be artefactual, due to differences in the thickness of the ground dentinal sections and due to the plane of sections of these specimens in relation to the amelodentinal junction. However, on SEM examinations, an amorphous, tubule free, mineralised layer was consistently seen on the dentinal aspect of the junction and dentinal tubules or odontoblast processes never extended through this amorphous layer to directly contact the enamel.

Structures termed enamel spindles that are believed to be fossilised odontoblast processes have been observed in human teeth (Boyde 1990) but no such structures were detected in the current study. A criticism of SEM examination is that it only allows the surface of the specimen to be viewed (Ten Cate 1994). However, in the current study, hundreds of SEM examination were made of both sectioned and fractured specimens and therefore it appears likely that if enamel spindles were present in equine teeth they would have been observed.

Decalcified dentine examined by light microscopy showed the dentinal tubules to contain shrunken, wavy structures, however, SEM examinations of these samples revealed odontoblast processes, circular in cross section, totally filling the lumina of the dentinal tubules. It was concluded that the structures seen filling the dentinal tubules on light microscopy of decalcified dentine may either have been staining artefacts or remnants of soft tissues such as the lamina limitant, collagenous fibres or an aggregation of denatured intertubular material as suggested by Torneck (1994).

Close to the amorphous tubule free layer at the amelodentinal junction, the dentinal tubules divided into several sub-branches. The presence of such branching indicates that equine odontoblasts produced several processes when dentinogenesis was initiated. With continuing dentinogenesis, these smaller processes merged to form

a single large odontoblast process. A similar picture has been noted in human teeth (Osborn 1981; Ferguson 1990).

Studies have shown that the peripheral and inner layers of human dentine are more sensitive to pain than its middle layer. The sensitivity of inner dentine can readily be explained as being due to its close association with the rich neural network of the pulp (plexus of Rashkow). However, many authors including Frank (1959), Tsatsas and Frank (1972) and Torneck (1994) noted that outer dentine contains virtually no nerve fibres. Brannstrom and Garberoglio (1972) and Tsatsas and Frank (1972) believe that the high sensitivity of this outer layer to pain may be mediated by hydrodynamic events within the dentinal tubules. For example, the traumatic opening of the amelodentinal extremity of a dentinal tubule may induce a change in the hydrodynamics of the tubule contents, which may in turn stimulate nerve endings present in the inner dentine (Tsatsas and Frank 1972).

The similarity of the dentinal tubular structures adjacent to the amelodentinal junction in human and equine dentine raises the question as to whether the equine amelodentinal junction is as sensitive as in humans. In horses unlike in humans, both primary and secondary dentine are major contributors to the normal occlusal surface of teeth and thus are directly and constantly exposed to the chemical and microbial challenges of the oral cavity and they additionally have to continuously withstand direct mechanical masticatory forces. These functional features casts doubt on whether equine dentine has any sensitivity at all. Question on the sensitivity of equine dentine to pain cannot be answered without physiological studies.

The diameter of dentinal tubules, and both the area and diameter of peritubular dentine increased from the amelodentinal junction to the junction of primary and

secondary dentine (medially), indicating that odontoblastic activity increases in this direction. In regular secondary dentine, peritubular dentine disappeared, but the increase in tubule diameters continued. However, this increase in dentinal tubule width should not be regarded as further evidence of increased odontoblast activity, since this increase was probably compensatory for the disappearance of peritubular dentine (Blake 1958; Takuma 1960; Fosse et al 1992).

The ratio of peritubular dentine to intertubular dentinal areas was measured at three different regions, (near the amelodentinal junction, in primary dentine close to the secondary dentine junction and in the regular secondary dentine). There were significant differences in this ratio between all three regions. However, there were no significant difference in this variable at different vertical levels in the same tooth or between different teeth.

Peritubular dentine was found to contain a higher proportion of inorganic material than intertubular dentine. Evidence for this comes from the SEM study of decalcified sections where peritubular dentine was found to be largely dissolved. This observation is also supported by the findings of Bradford (1963), Takuma et al (1966) and Kierdorf and Kierdorf (1992). The presence of a high proportion of inorganic components in peritubular dentine increases the wear resistance of such dentine (Bradford 1963; Kierdorf and Kierdorf 1992). Highest amounts of peritubular dentine and thus maximum wear resistance were found at the junction of primary and secondary dentine, i.e. half way between the amelodentinal junction and pulp. It would be therefore expected that a raised area of dentine would be found on the occlusal surface at this site. Likewise, as the dentine near the amelodentinal junction

contains lower amounts of peritubular dentine, it therefore has least wear resistance and it should theoretically wear faster at this site than in the mid dentinal region.

In practice however this does not happen, as the dentine near the amelodentinal junction is protected from high rates of wear by its proximity to enamel and it is the dentine mid way between the enamel and the pulp that is most vulnerable to excessive wear. Hence the presence of high amounts of wear resistant peritubular dentine in the latter area. Secondary dentine wears faster than dentine near the amelodentinal junction and hence a hollow develops on the occlusal surface at the secondary dentine.

Some early studies indicated that human peritubular dentine developed after tooth eruption in response to noxious stimuli (Blake 1958; Johanson & Parks 1962). This theory has largely been disregarded after the discovery of peritubular dentine in both unerupted and recently erupted teeth, both brachyodont and hypsodont (Fosse et al 1992; Kierdorf and Kierdorf 1992). The present study also revealed the presence of peritubular dentine in unerupted equine teeth. It could also be argued that if peritubular dentine was only formed in response to external stimuli, highest amounts of it would occur close to the exterior of the tooth, but in practice this does not occur. Additionally, the regular appearance of peritubular dentine is further evidence that it is part of normal physiological dental development, because its development in response to external influences would be expected to lead to irregular deposition, primarily at the sites of insult.

The mechanism of development of peritubular dentine is unclear. Torneck (1994) has suggested three possible mechanism. One proposal is that peritubular dentine is formed by mineralisation of the organic material found within the dentinal

tubules and this theory is also supported by Ten Cate (1994). This mineralisation is thought to occur by passive redistribution of mineral ions from intertubular dentine to this organic tissue. However, neither author has explained how mineral ions could move passively from the low mineral density intertubular dentine to the higher mineral density peritubular dentine. Intertubular dentine itself has a requirement for mineral ions as it is incompletely mineralised. Therefore, the passive transfer of ions from the intertubular dentine to peritubular dentine appears unlikely.

The current study supports Torneck's (1994) second theory which proposes that odontoblast processes are responsible for the deposition of peritubular dentine. Examination of developing equine dentine in this study has not shown peritubular dentine within predentine or recently mineralised dentine. As the deposition of peritubular dentine occurs behind the mineralisation front of the dentine consequently it is not found in predentine. Whilst it is likely that the odontoblast cell bodies are not directly involved in peritubular dentine formation, the intimate relationship between the odontoblast **processes** and peritubular dentine seen on SEM examination in this study suggests that the odontoblast processes may be.

Differences in the structure and organisation of equine intertubular and peritubular dentine were revealed by the decalcification and acid etching processes. Intertubular dentine was largely unaffected by decalcification or etching and thus very little of its internal structure was visible on microscopy. These findings suggest that their mineral and organic components were tightly bound together, with the strong bond between the two components providing protection against the above noted processing techniques. Decalcification removes the mineral component of tissues and if the minerals are only loosely bound, they are more readily lost. In the case of

intertubular dentine, neither its inorganic or organic constituents were removed during decalcification. In contrast, peritubular dentine readily lost its mineral components during decalcification to reveal a well organised network of collagenous fibres.

It would therefore appear that a much looser association exists between the organic and inorganic components of peritubular dentine. Indeed, its mineral component may have been laid down independently of the organic material, with few or weak bonds between them. Following acid etching, small channels were seen extending peripherally from the centre of the dentinal tubules. These are possibly lateral branches of dentinal tubules that carry branches of the odontoblast processes, or the sites of collagen fibre bundles or a combination of both.

Examination of decalcified and acid etched dentine showed a distinct relationship between the proportion of intertubular to peritubular dentine and the texture of the intertubular dentine. Where the proportion of intertubular dentine to peritubular dentine was high, the intertubular dentine had a open and rough texture. As the relative amounts of intertubular dentine decreased, the intertubular dentinal texture becomes compact and smooth. It is unclear if the chemical composition of rough and smooth intertubular dentine differs. However, it is interesting to speculate that the difference in texture is due to physical pressures exerted by peritubular dentine on the adjacent less mineralised intertubular dentine.

Several difference were found between primary and secondary equine dentine in this study, including in the sizes and directions of the dentinal tubules and in the presence of peritubular dentine. Due to the absence of peritubular dentine in secondary dentine, its dentinal tubules were much wider than in primary dentine. The dentinal tubules also showed a distinct change of direction at the junction of primary

and secondary dentine, as also reported in human dentine (Scott and Nylen 1966; Jones 1990).

The disappearance of mineral rich peritubular dentine at the boundary of primary and secondary dentine is reflected in the gross appearance of the occlusal surface. Primary dentine that contains peritubular dentine had an almost translucent appearance similar to enamel, as both are very highly mineralised. In contrast, secondary dentine has a dull opaque appearance. Additionally, at the occlusal surface, secondary dentine absorbs pigments from food which gives it dark brown colour and hence, occlusal secondary dentine is readily identified with the naked eye. As previously noted (Goody 1983) the discrete, brown incisor secondary dentine is known as the "dental star" and was formerly thought to have great significance in ageing horses.

As the presence of peritubular dentine confirms mechanical advantages to dentine it is unclear why it is absent from secondary dentine. There are several possible explanations for this fundamental difference between primary and secondary dentine. It is possible that due to continuous dentine deposition, the pulp cavities gradually become smaller and consequently the odontoblasts become progressively more crowded. This may cause the odontoblast to expend much of their energy in retreating medially rather than in depositing peritubular dentine. Additionally, during the formation of secondary dentine the odontoblasts become even more crowded and consequently some die. The death of some odontoblasts may influence the activity of the remaining cells and could inhibit the formation of peritubular dentine. The initial deposition of secondary dentine coincides with the completion of root dentine formation and with the initiation of eruption (Kierdorf & Kierdorf 1992; Torneck

1994). Dental eruption is a complex and traumatic phenomenon requiring major changes in the tooth and surrounding tissues (Boyde 1990) and the physical and chemical factors that stimulate the deposition of peritubular dentine may be lost following tooth eruption.

A further explanation may be that the disappearance of peritubular dentine could be due to reduced odontoblast activity due to the ageing process. EM examinations have shown that odontoblast processes undergo three different phases during their life cycle. In each period they have a characteristic shape that is clearly associated with their degree of activity. In their final period, they largely lose their cytoplasm and intracellular organelles (Ten Cate 1994) and these intracellular alterations may be associated with a cessation of peritubular dentine secretion.

Bradford (1963), Takuma et al (1966) and Kierdorf & Kierdorf (1992) all reported that the presence of large amounts of peritubular dentine is a distinct characteristic of equine dentine. However, the present study found peritubular dentine confined to primary dentine. Regular secondary dentine forms a very significant contribution to the total amount of equine dentine and therefore, the statement of the above authors regarding this "characteristic" of equine dentine should be qualified.

This study has also shown that the sizes and numerical density of dentinal tubules depends on their site within the tooth. No significant difference was recorded in the size and density of dentinal tubules from the same regions at different vertical levels within the tooth or between different teeth. Similar measurements of dentinal tubules at three similar locations in the dentine of humans, monkeys, rats, cats and dogs (Forsell-Ahlberg et al 1975) showed no significant difference between species. In contrast, Hildebolt et al (1986) found these measurements to be species specific. In

the present study, the consistency of findings from the same sites within a tooth, between different teeth and between different horses suggests that dentinal tubules measurements could be a useful parameter for assessing the taxonomic relationships of *Equus caballus* to other species.

This study showed that the density of dentinal tubules in equine primary dentine was similar to other species including humans, baboon and the dog (Ketterl 1961; Garberoglio & Brannstrom 1976; Fosse et al 1992). However, equine regular secondary dentine contained much higher numbers of dentinal tubules/unit area than the dentine of other species. This apparent difference may be due to the fact that dentinal tubules may only have been examined in primary dentine in these other brachyodont studies.

An interesting observation was that the location of the dentinal tubules within equine peritubular dentine varied between different sites in the tooth. This finding was also observed in deer and wild boar dentine by Kierdorf & Kierdorf (1992). The dentinal tubules were located peripherally within the peritubular dentine in dentine located close to the pulp cavities and deep to the cusps, but not where it was located deep to the infundibula (of the upper cheek teeth). As the odontoblast cell bodies become crowded during the deposition of secondary dentine, the regularity of position of the odontoblast processes was lost. These results indicated a change in the direction of mineral and matrix secretion across the membrane of the odontoblast process. In some areas, the secretion appears to have been uniform around the circumference of the odontoblast processes, leaving it centrally positioned in the peritubular dentine, whilst in other regions of primary dentine, the secretion of peritubular dentine components occurred more through one side of the membrane,

thus leaving the odontoblast process situated peripherally in peritubular dentine. It is interesting to speculate on the factors that influence the secretion of peritubular dentine along the length of the odontoblast processes, including possible genetic, mechanical or nutritional influences.

Significantly more odontoblast processes were present in primary than in secondary dentine. This may be related to the presence of peritubular dentine within the primary dentine with the physical link between peritubular dentine and the odontoblast processes providing protection to the odontoblast processes during the preparation of specimens for microscopy. As no peritubular dentine was found in secondary dentine and additionally no fibrous attachments existed between the odontoblast processes and the dentinal tubules in secondary dentine, their unattached odontoblast processes may be more vulnerable to rupture and contraction during processing as compared to their counterparts in primary dentine.

The current study showed that the lumina of most dentinal tubules on the occlusal surface remain open. Additionally, almost all tubules of both primary and regular secondary dentine that were exposed on the occlusal surface contained remnants of odontoblast processes. It is surprising that these open tubules were present on the occlusal surface of dentine in direct contact with the oral environment, as theoretically this should predispose this dentine to chemical and microbial insults. However, examination of the occlusal surface of these teeth did not show any pathological changes in the occlusal dentine. The occlusal surface of untreated specimens was found to be covered by a thick, acquired organic pellicle and this pellicle may have helped seal the entrances of the dentinal tubule lumina, preventing the entry of chemicals and microorganisms Even if microorganisms could enter the

dentinal tubules they could not reach the pulp cavity because the occlusal aspect of the pulp cavities was completely sealed off by the presence of a layer of irregular secondary dentine in which all the tubule lumina were fully obliterated (sclerosed). This would imply that the exposed odontoblast processes were not viable and thus calcification of dead odontoblast processes appear to be only rational explanation for the presence of exposed odontoblast processes on the occlusal surface.

Light microscopic examination of transversely sectioned, decalcified, stained equine dentine showed the presence of two types of incremental lines, which could be readily distinguished from each other by their appearance and orientation. The appearance of these incremental lines is believed to be related to the plane of sectioning of specimens relative to the longitudinal axes of the dentinal tubules. On transverse dental sections, thin incremental lines were inconsistently present. As these lines are formed by parallel interdigitations of dilatations and constrictions of neighbouring dentinal tubules, they are only visible if the plane of section is approximately parallel to the long axes of the tubules. When the plane of section relative to the long axes of dentinal tubules changes from longitudinal to transverse, these thin incremental lines consequently disappear. In this study, most dentinal tubules were found to be oriented almost parallel to the long axis of the teeth. In transverse sections of teeth longitudinally sectioned tubules were limited to the dentine found between ridges and invaginations of enamel in the lower cheek teeth. Therefore, it is unlikely that consistent, complete incremental lines would be observed in most transverse sections.

The thin incremental lines had a consistent appearances in decalcified, H & E stained sections, with areas between incremental lines staining a paler colour than the

incremental lines. The variation in staining properties between the incremental and interincremental areas indicated a possible difference in chemical composition or in the orientation of collagenous fibres between these two areas. In human dentine, incremental lines caused by rhythmic daily (circadian) growth variation cause constrictions and dilatations of dentinal tubules that are termed von Ebner incremental lines. However, some authors including Shellis (1981) and Schroder & Frank (1985) believed that von Ebner incremental lines are due to diurnal changes in the orientation of organic fibrils between successive layers rather than from alternating diurnal phases of activity and quiescence of odontoblasts.

Torneck (1994) reported the presence of a regular daily (circadian) and additionally, a 5 day cycle of rhythmic activity in human odontoblasts, which can be differentiated by the thickness of their respective incremental lines. Torneck suggested that the daily incremental lines are insignificant and should not normally be noted and that the incremental lines described by Jones (1990) as von Ebner incremental lines actually represent 5 day cycles, rather than daily cycles.

The second type of incremental lines arises from disturbances of dentinogenesis caused by dietary, systemic or environmental stresses. Poor nutrition leading to mineral deficiency will disturb the mineralisation of dentine, as will systemic diseases and the administration of certain drugs such as tetracyclines (Shellis 1981; Torneck 1994). Shellis (1981) termed the lines caused by drugs as chemicotramatic lines. Thick incremental lines were consistently present in transverse sections of equine dentine. The pattern of these lines were thought to be useful to trace the history and developmental of an individual teeth (Boyde 1990).

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CHAPTER 6

GROSS, LIGHT MICROSCOPIC AND ULTRASTRUCTURAL EXAMINATION OF CEMENT

RESULTS

6.1. Qualitative findings

The surfaces of recently erupted equine teeth were fully covered by a layer of cement (figs 4 & 7). With normal attrition, this soft occlusal cement layer and thin enamel layer were quickly and fully worn away, dividing the cement of the upper cheek teeth and incisors into peripheral and infundibular cement and thus exposing the secondary (and main) occlusal surface of these hypsodont teeth. In the lower cheek teeth, just peripheral cement remained at the secondary occlusal surface. The thickness of peripheral cement of both upper and lower cheek teeth varied greatly, depending on the degree of folding of the underlying enamel (figs 10 & 13). Macroscopic observations of 17 transversely and 2 vertically sectioned and 2 fractured upper M1s and PM4s in the current study showed 5 cases (23.8%) of caries, with 4 involving the centre and one the periphery of infundibula. In all 4 cases the caries was confined to the cement and the infundibular enamel was unaffected.

64.7% of normal infundibula (without caries) of upper cheek teeth had one or two small, round or triangular shaped channels located in their centre that were filled with shrunken connective tissue (figs 81, 82 & 83). Examination of sections at different vertical levels of the teeth showed that these central channels extended corono-apically to variable depths and generally narrowed towards the apex. Infundibular cement of recently erupted teeth contained central vascular channels with branches extending towards the periphery of the infundibular cement. These channels

branched extensively and gradually reduced in size towards the periphery (fig 84). In fractured specimens of recently erupted teeth the walls of vascular channels were smooth and covered with a shiny gelatinous structure (fig 84) and the lumina of these vascular channels contained organic debris (fig 85), but in fully erupted teeth these vascular channels were empty.

The vascular channels of infundibular cement were spaced a median distance of 118.91 μm (range 51.8-274.48 μm) apart and their diameters ranged from 18.6 to 181.91 μm (median 40.75 μm). The number of vascular channels/0.026 mm^2 of cement ranged between 17-34 in normal cement to 19-44 in hypoplastic cement. The infundibular vascular channels occupied up to 31 % of the total cemental volume in the cement surrounding defects.

The cement which filled the peripheral enamel invaginations in the lower cheek teeth contained vascular channels oriented almost perpendicular to the occlusal surface (fig 86). The vascular channels of both peripheral and infundibular cement normally terminated close to, and seldom reached the amelodentinal junction. The median distance between the termination of vascular channels and the amelodentinal junction was 36.74 μm (range 10.04 to 97.28 μm).

Light microscopy showed all decalcified cemental sections to contain lacunae, mainly oval or circular, but occasionally triangular or rectangular in outline (fig 87) and lacunae of several different shapes could be seen in any one particular area (fig 88). The walls of lacunae contained small holes that represented the entrances of canaliculae (figs 89 & 90) and in some sections, the lumina of lacunae were seen to be continuous with those of canaliculae (fig 91). SEM examinations showed the peripheral regions of some lacunae to be surrounded by a highly mineralised layer

mineralised layer which appeared to be more compact in structure than the remaining cement (fig 90). The walls of the lacunae were markedly irregular in TEM sections. The sizes of canaliculae varied greatly (median diameter 0.44 μm, 0.2-1.5) and the smaller ones could best be differentiated at high TEM magnifications (figs 91 & 92).

Light microscopy of undecalcified cemental sections showed the presence of spider-like cementoblasts near the developing front of peripheral cement (fig 93). Unlike cementocytes, these cementoblasts, completely filled the lacunae and had thin cytoplasmic extensions radiating out beyond the boundaries of the lacunae into canaliculae and intermingling with cytoplasmic extensions of neighbouring cementoblasts (fig 93). These cytoplasmic extensions were usually oriented toward the developing front of cement (fig 93). SEM examination of the surface of infundibular cement of a recently erupted teeth showed numerous minute prominences of different shapes (fig 94). When SEM magnification was increased, these prominences were identified as cementoblasts embedded in mineralised cement (fig 94). Extensions radiated out from these prominences and intermingled with those of adjacent cementoblasts (fig 94).

In decalcified sections of cement, most lacunae contained one, or occasionally two small, shrunken cementocytes. They were located at the sides of the lacunae in all areas of cement. SEM examination showed that these cementocytes were attached to the walls of the lacunae by their calcified cytoplasmic processes (fig 95).

6.2. Cemental fibres

SEM examination of decalcified sections showed the lacunae to contain a network of collagenous fibres (fig 89). TEM examination showed that some of the collagenous fibrils in interlacunar areas were oriented randomly in a branched

and others in parallel bundles (figs 91, 92 & 96). Sections that were cut parallel to the developing front of peripheral cement contained collagen fibres (median 62 nm, 33-90 nm thick) that were obliquely or longitudinally sectioned and that had ribbon-like shapes (fig 96). The intrinsic fibres of peripheral cement were often oriented at right angles to the developing front of cement.

Extrinsic fibres were present only in the peripheral cement and were small and round in transverse section. They were oriented at right angles to the intrinsic fibres, i.e. were oriented parallel to the developing front of cement. They ran both as single fibres and in tightly packed bundles (figs 91 & 96). Most of the single extrinsic fibres scattered throughout the peripheral cement were surrounded by semicircular or circular shaped spaces (fig 96) that represented highly mineralised areas. On low power TEM magnification, the tightly packed bundles of extrinsic fibres, also known as Sharpey's fibres, appeared as distinct round structures in the peripheral cement. They had diameters of between 0.9-55 μm (median 2.5 μm) (fig 91) and were surrounded by intrinsic collagenous fibres oriented perpendicular to them (fig 96). TEM examination showed some of the extrinsic fibres that formed Sharpey's fibres to be surrounded by thin empty spaces (fig 96).

6.3. Cemental hypoplasia

Two types of cemental hypoplasia were found in equine cheek teeth. The first, which was termed **junctional cemental hypoplasia** appeared as spaces varying from focal (fig 97) to long narrow defects along the amelocemental junction (fig 98). These defects were found at the amelodentinal junction of both peripheral and infundibular cement. The boundaries of these defects were formed by enamel and cement and the cement adjacent to such defects had a normal appearance (figs 97 & 98).

The second type of cemental hypoplasia of cheek teeth termed **central infundibular cemental hypoplasia** was confined to the middle region of infundibular cement (in upper cheek teeth only) and varied greatly from the first type in the shape, size and content of the hypoplastic spaces. In some upper cheek teeth, large defects extended from the occlusal surface to the apical aspect of the infundibular enamel (figs 99 & 100). These central infundibular defects sometimes merged with the previously noted junctional cemental hypoplasia (fig 99). In some teeth, large irregular central defects decreased in size towards the infundibular apex, while in others, the defect increased in size apically. In other teeth, the occlusal aspect of infundibular cement appeared intact, but its apical region contained large cemental defects.

In recently erupted cheek teeth, the central hypoplastic infundibular spaces were filled with soft connective tissue and blood vessels derived from the dental sac (fig 85). The cement adjacent to such hypoplastic areas was very porous and contained large vascular channels (figs 85, 99 & 100). When the coronal aspect of the cemental infundibular cemental defect was large and its lumen was exposed to the oral cavity this connective tissue was found to be degenerate and food particles were also found in this area (fig 100). In contrast, when the occlusal cemental surface was intact and the hypoplastic areas were deep in the infundibula and thus had no connection to the oral cavity, their soft connective tissue contents appeared shrunken but undegenerated (fig 99). Light microscopic examination of ground and decalcified sections of the bulky peripheral cement of lower cheek teeth showed the presence of incremental lines that had irregular courses and differing thicknesses. The intervals between successive incremental lines varied, and occasionally, adjacent incremental lines merged along their course (fig 101).

6.4. Quantitative findings

6.4.1. Lacunae diameter

Data relating to lacuna diameters were normally distributed and are presented as mean (±SD). Cemental lacunae were wider in infundibular than in peripheral cement, and in peripheral cement, were greater in the upper than in the lower cheek teeth (appendix 20) and these features are diagramatically presented fig 102.

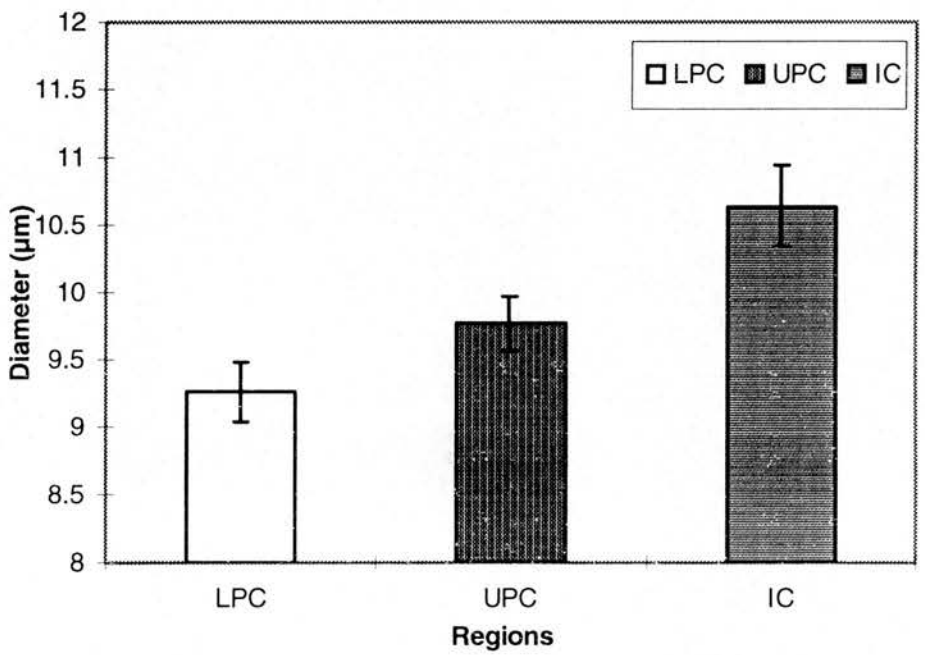

Fig 102: Diameters (mean and standard deviation) of lacunae in lower peripheral cement (LPC), upper peripheral cement (UPC) and infundibular cement (IC) of PM4 in 7 horses.

In upper cheek teeth, the mean diameter of lacunae in vertical level 1 was significantly greater than in levels 2 and 3 (**tables 23 & 24**). However, in the lower cheek teeth, the mean values for level 2 were non-significantly lower than for levels 1 and 3 (**tables 23 & 25**).

Levels	Upper PM4		Lower PM4		Upper and lower PM4	
	Mean	SD	Mean	SD	Mean	SD
1	10.55	2.36	9.64	2.23	10.33	2.36
2	9.83	2.2	8.88	1.86	9.49	2.13
3	9.78	1.98	9.24	2.05	9.6	2.02

Table 23: Diameter (μm) of lacunae [mean and standard deviation (SD)] of at three levels of upper and lower PM4

Analysis of variance confirmed that these lacunae diameters differed significantly between different levels and regions (upper cheek teeth only), different teeth (upper and lower) and different horses (**tables 24, 25 & 26**).

Source of variation	Degree of freedom	Sum squares	Mean squares	F Ratio	Probability
Levels	2	87.84	43.92	9.18	<0.000
Error	697	3335.23	4.79		
Regions	1	167.03	167.03	35.81	< 0.000
Error	698	3256.04	4.66		
Horses	6	206.93	34.49	7.43	<0.000
Error	693	3216.14	4.64		

Table 24: Analysis of variance on lacunae diameter of cement of upper PM4 of 7 horses at different levels, regions and in different horses

Source of variation	Degree of freedom	Sum squares	Mean squares	F Ratio	Probability
Levels	2	27.90	13.95	3.42	0.034
Error	320	13.03.89	4.07		
Horses	6	95.64	15.94	4.07	<0.001
Error	693	3216.14	4.64		

Table 25: Analysis of variance on lacunae diameter of cement of lower PM4 of 7 horses at different levels, regions and in different horses

Source of variation	Degree of freedom	Sum squares	Mean squares	F Ratio	Probability
Levels	316	10.335	70.00	14.92	<0.000
Error	368	9.485	4.69		
Regions	1	304.42	304.42	67.25	< 0.000
Teeth	1	171.08	171.08	36.73	<0.000
Error	1021	4754.86	4.66		
Horses	6	265.69	4.28	9.65	0.000
Error	1016	4660.24	4.59		

Table 26: Analysis of variance on lacunae diameter on combined values for cement of upper and lower PM4 of 7 horses at different levels, regions and in different horses

6.4.2. Density of lacunae per unit area

The number of lacunae/0.15 mm^2 area of cement in different regions and different levels of 7 individual horses is presented in appendix 21 and combined values of these data in different regions and levels are respectively given in **tables 27 & 28**.

Regions	Upper PM4			Lower PM4			Upper and Lower PM4		
	Median	Min.	Max.	Median	Min.	Max.	Median	Min.	Max.
1	19	11	25	18	12	23	18	11	25
2	16	10	25	-	-	-	-	-	-

Table 27: Numbers of lacunae/0.15 mm^2 area at two regions [peripheral (1) and infundibular (2)] of upper, and one region (peripheral) of lower PM4

Levels	Upper PM4			Lower PM4			Upper and Lower PM4		
	Median	Min.	Max.	Median	Min.	Max.	Median	Min.	Max.
1	16.5	11	25	15	12	19	16	11	25
2	17.85	11	25	19.29	17	21	19	11	25
3	17.31	10	25	18	17	23	18	10	25

Table 28: Number of lacunae/ 0.15 mm^2 area at three levels of both upper and lower PM4

As data were non-normally distributed statistical analysis of measurements of numbers of lacunae/unit area was made using Kruskal-Wallis and Mann-Whitney tests. Despite the presence of significant differences in the diameter of lacunae, the number of lacunae per unit area did not significantly differ between different levels, regions, horses and teeth (**table 29**).

Variables		median	Range	Statistic	Comments
Horses no	7	18	11-25		
	8	13.5	13-25	H=2.92	
	9	19	12-23	d.f.= 6	9=10=14>7=15>8
	10	19	14-22	P= 0.818	Non-significant
	11	17	13-22		
	14	19	11-25		
	15	18	16-25		
Levels	1	16	11-25	H=2.11	2>3>1
	2	19	11-25	d.f.= 2	Non-significant
	3	18	10-25	P=0.349	
Teeth	Upper	17	10-25	W=1152.5	Lower>Upper
	Lower	18	12-23	P= 0.6728	Non-significant
Regions	1	18	11-25	W= 1234.5	1>2
	2	16	10-25	P= 0.1644	Non-significant

Table 29: Comparison of number of lacunae/0.15 mm^2 at different vertical levels, different sites and in different teeth in 8 horses

6.4.2. Lacunae area:total cemental area

The ratio of lacunae area:total cemental area ranged between 0.5-1.4 (median 1) in peripheral cement of the upper cheek teeth, 0.7-1.2 (median 0.85) in peripheral cement of lower cheek teeth and 0.5-2.1 (median 1.1) in infundibular cement (upper cheek teeth) (appendix 22). The ratio of lacunae: total cemental volume in levels 1, 2 and 3 was respectively 1.1, 0.9 and 1 in upper and 0.7 , 0.9 and 0.9 in lower cheek teeth. Statistical examination of these data showed no significant differences in this variable between different regions in the horizontal plane or between vertical levels of upper and lower cheek teeth cement.

6.2. DISCUSSION

The current study has shown cemental lacunae to be distributed in a random pattern within cement. Consequently, it was difficult to measure the true size of lacunae, as some were sectioned in their middle, but others tangenitally, close to their periphery. Therefore, the recorded variation in the diameters and also in the shapes of lacunae are believed to be partially due to different planes of sectioning. However, these sectioning artefacts should have just a limited effect on results because large numbers of lacunae were examined at each site.

The lacunae of infundibular cement (upper cheek teeth) were found to be larger than those of upper cheek teeth peripheral cement, which in turn were larger than those in lower cheek teeth (peripheral) cement. As lacunae size is believed to be determined by the cementoblast size, the cementoblasts of infundibular cement should be bigger than those of peripheral cement in both upper and lower teeth. It is unclear whether these variations in cementoblast size have any significance on the development and function of equine cement.

Despite significant difference between lacunae diameters at the different sites (table 26), the number of lacunae/unit area (table 29) and the ratio of lacunae area: total cemental area did not significantly differ between different regions, levels or teeth. This indicates that all cementoblast secrete the same amount of extracellular substances and are responsible for territories of equal size.

Much variation was found in the shapes of lacunae in equine cement. Jones and Boyde (1974) reported the shape of equine cemental lacunae to be dependant on the site of cement, being circular adjacent to the amelodentinal junction and long and narrow in the outer layer of coronal (peripheral) cement. However, in the current

study, lacunae of different shapes were seen in all cemental areas and as noted, the recorded variations in shape were believed to be partially due to different planes of sectioning. Additionally, serial examination of many sections of cement from the amelocemental junction to its developing front did not reveal any specific trends in lacunae shape.

Lacunae were usually separated from the amelocemental junction by a very thin acellular band of cement. This is believed to be formed from the extracellular matrix secreted by cementoblasts lying adjacent to amelocemental junction and should not be considered as being the equivalent of acellular human root cement.

Light microscopic examinations of decalcified cemental sections inconsistently showed the presence of incremental lines in thick peripheral cement adjacent to the periodontal ligaments of the lower cheek teeth. This finding indicates that equine coronal cement is not deposited in a regular incremental pattern. Selvig (1965) and Osborn (1981) found incremental deposition of root cement in human teeth and believed these incremental lines to be due to different orientation of intrinsic fibres between successive lines of cement (Osborn 1981). Microradiographic examination of human teeth has also shown variation in the mineralisation of incremental lines between the cementodentinal junction and the developing front of cement (Selvig 1965).

The orientation of the extrinsic and intrinsic fibres in equine cement found in the current study are in agreement with the findings of Jones and Boyde (1974). There is, however, disagreement between the two studies concerning the sizes of these fibres, particularly of Sharpey's fibres. Jones and Boyde recorded the diameters of intrinsic fibrils to be between 1-2 μm and the diameters of Sharpey's fibres to be

between 5-10 μm, whilst in the current study, values of 0.33-0.90 μm (median 0.62 μm) and 0.9-5.5 μm (median 2.5 μm) respectively, were found. These variations may be due to differences in the number of fibre measurements between the two studies and also to the chosen criteria for measurements. For example, Jones and Boyde (1974) measured the maximum lengths of lacunae in a plane parallel to the developing front of cement, whereas in this study the boundaries of lacunae were measured in transverse sections of cement.

Sharpey's fibres are formed by dense bundles of extrinsic fibrils and in fully decalcified cemental sections in this study, they were found to be surrounded by ground substance that contained a network of intrinsic cemental fibrils. In the present study, empty spaces were not observed around Sharpey's fibres as have been reported by Jones and Boyde (1974) who regarded their presence as evidence of the presence of a highly mineralised layer around Sharpey's fibres. Since, some sections examined in the current study were fully decalcified, any highly mineralised areas would therefore have appeared as empty spaces, and no such areas were identified in this study.

The present study has shown that in hypoplastic infundibular cement, vascular channels occupied up to 1/3rd of the residual cemental volume. As the occlusal aspects of these defects were usually in direct contact with the oral cavity, microorganisms and their products could easily spread from the central channel along the lumina of the vascular channels that extended throughout infundibular cement, thus causing widespread dissolution of the cement, i.e. caries. Although caries was found in 5 out of 21 upper cheek teeth, it remained localised to the infundibular cement. The lumina of the vascular channels taper towards the periphery of

infundibular cement and additionally the lumina of these vessels are smaller in normal than in hypoplastic cement. These characteristics may prevent food particles (an ideal medium for microorganisms) from reaching the periphery of cement and this possibly reduces the risk of diffuse infundibular caries. As noted, vascular channels of normal equine cement were narrower than those of hypoplastic cement, indicating that with progressive cemental deposition, the lumina of blood vessels reduce in size.

Infundibular vascular channels were commonly found to terminate adjacent to the amelodentinal junction with an avascular region existing between infundibular cement and enamel. This avascular region may also enhance the resistance of peripheral infundibular cement to caries and in turn protect the adjacent infundibular enamel against caries. The results of the present study indicates a relationship between the volume of the cemental defect and the development of gross infundibular caries. The greater the volume of hypoplastic cement, the larger the amounts of residual connective tissue which may predispose to infundibular caries. The connective tissue of hypoplastic cement may lead to caries in two different ways. Firstly, as the tooth erupts the blood supply to this connective tissue is lost, resulting in ischemic necrosis. These ischemic tissues may release acidic necrotic products that may lead to cemental caries. Additionally, this necrotic tissue is an ideal medium for microorganisms from the oral cavity which could also cause cemental caries. Consequently, it is suggested that the initiation of infundibular caries may not only be due to the effects of acidic by-products of microbial digestion of food particles as suggested by Baker (1974), but also from the necrosis of connective tissue.

The relationship between hypoplastic areas of cement and the occlusal surface of teeth is also important in the development of infundibular necrosis. In teeth, where

the lumen of hypoplastic cement was not in direct communication with the oral cavity, the hypoplastic spaces only contained shrunken connective tissue, and the cement surrounding such hypoplastic spaces appeared normal. Therefore, the development of infundibular necrosis also appears to depend on the oral microorganisms having direct access to the hypoplastic cement. However, with age and normal attrition, these hypoplastic areas would eventually be exposed to the oral cavity.

Junctional cemental hypoplasia was located along the amelodentinal junctions and may result from failure of disintegration of the reduced enamel epithelium or failure of resorption of the enamel surface. Jones and Boyde (1974) suggested that this type of cemental hypoplasia could be due to excessively rapid deposition of cement and was more common in incisors than in cheek teeth.

The cause of central infundibular cemental hypoplasia may be due to premature eruption of teeth, premature removal of the overlying deciduous tooth ("cap") by veterinarians or owners, or premature occlusion of its central vascular channel. With premature tooth eruption, the blood supply to the connective tissue and cementocytes is completely lost and no further cement deposition can occur. On the other hand, premature deposition of coronal infundibular cement could obstruct the blood supply to connective tissue in the infundibulum leading to cemental hypoplasia of deeper levels. Central infundibular cemental hypoplasia (defect) was present in 43% of both deciduous and permanent equine upper cheek teeth and was suggested to be the main cause of generalised dental caries in horses (Baker 1974; Baker 1979). However, Wafa (1988) found 30.7% of equine teeth had infundibular caries but generalised dental caries was only present in 2.8 % of such cases. Consequently,

Wafa suggested that equine dental caries is usually a benign disorder. This area merits further study.

CHAPTER 7

AMELOCEMENTAL JUNCTION

RESULTS

Equine peripheral cement was found to be deposited both directly and indirectly on the surface of enamel. When indirectly deposited, the cement was separated from enamel by a thin, smooth, mineralised acellular layer, which could be readily separated from enamel, particularly in dried dental sections (fig 103). When directly deposited, cement was laid down on either an unresorbed or resorbed enamel surface. The surface of unresorbed enamel had a honeycomb-like pitted appearance, with the bottoms of these pits formed by enamel prisms and the pit walls by interprismatic enamel (figs 104 & 105). These pits were divided into two types, depending upon their transverse shapes and depths. The most common type of pits were shallow, had a rounded shape and contained semicircular or crescent shaped ridges near their bases (fig 105). The less common, second type of pits were deeper, had an almost rhomboidal outline and were surrounded by large amounts of interprismatic enamel (fig 105).

The cemental surface of resorbed enamel contained depressions of variable shapes and sizes, and both prismatic and interprismatic enamel could be identified within these depressions (fig 106), with interprismatic enamel appearing as thin ridges around the prisms (fig 106). In areas where a number of these enamel depressions touched, their boundaries became less pronounced and a number of these adjoined depressions contained irregular ridges and depressions (fig 104). At low power SEM magnification, the cemental surface of enamel showed both focal and

diffuse areas of resorption and this resorption was more marked on the cemental surface of infundibular than peripheral enamel.

7.2. DISCUSSION

Where indirect cemental deposition occurs, a thin mineralised structureless layer that represents the reduced enamel epithelium remains between the cement and enamel. This layer represents the reduced enamel epithelium that remained unresorbed during cementogenesis. The deposition of cement on the surface of reduced enamel epithelium in the horse may be similar to the deposition of cellular cement on the surface of acellular cement in human teeth (Jones and Boyde 1974). In the current study, the ease of separation of cement from enamel in dried specimens, indicates that a weak connection is present between these tissues with this type of cemental deposition. A similar finding has been reported in bovine coronal (peripheral) cement by Mills and Irving (1967). Therefore, it is assumed that resorption of the reduced enamel epithelium may be required not only for the induction of cementogenesis as suggested by Jones and Boyde (1974), but perhaps more importantly, to allow the establishment of a strong bond between these two tissues (Kawai 1955).

The current study found that where reduced enamel epithelium had disintegrated, i.e. in direct cemental deposition, the enamel surface could be resorbed or unresorbed. The surface of unresorbed enamel had a honeycomb like appearance due to the presence of numerous pits formed by prismatic and interprismatic enamel. These pits were divided into two types according to their depth, and to their shapes in the transverse plane, with both types of pits being separated by thick areas of interprismatic enamel. Therefore, the differences between these pit types can be

appearance of both pit types corresponds to that of eq. type 3 enamel, with prisms represented by pits, completely surrounded by thick areas of interprismatic enamel.

The presence of a pitted appearance of the surface of unresorbed equine enamel has previously been reported by Jones and Boyde (1974). However, these authors noted the presence of two types of enamel pits on the surface of unresorbed enamel at the amelodentinal junction, with one of their types encompassing both types of pits described in this study. Their other pit pattern corresponds to eq. type 1 enamel (radial enamel) which was found at the amelodentinal junction but never at the amelocemental junction in this study.

The smooth and compact structure of the unresorbed enamel surface observed in the present study was different to the granular appearance of the unresorbed equine enamel surface described by Jones and Boyde (1974). They claimed that this granular appearance was due to incomplete enamel mineralisation. Differences in sample preparation techniques between Jones and Boyde's and the current study are probably responsible for these different findings. Jones and Boyde used a freeze-fracture technique to prepare their samples and a common artefact with this technique is condensation of tissue fluid during the processing. It is therefore probable that the granules seen by Jones and Boyde (1974) were caused by this artefact, that does not occur during the drying technique used in the present study.

Granules were observed on the developing enamel front in embryonic but not in adult teeth in the current study. During maturation, enamel undergoes several phases of exchange of mineral and organic matrix between its developing front and ameloblasts (Suga 1979). As the ameloblasts become less active and eventually die,

the withdrawal of organic matrix from the developing enamel surface remains incomplete.

The surface of resorbed enamel was composed of pits of variable shapes and sizes. The presence of such pits on the cemental interface of equine enamel was previously noted by Kawai (1955) and Jones and Boyde (1974) and in elephant enamel by Kawai (1955) and Schmidt & Keil (1971). The latter authors have termed these pits "resorption bays" as they believed they were due to resorption of the enamel surface. Both Schmidt & Keil (1971) and the current study have shown these resorption bays to cover all of the enamel surface and they substantially increase the area of contact between cement and enamel. It was suggested by Jones and Boyde (1974) that the deposition of peripheral cement in the horse depended on enamel resorption. This proposal is not supported by the current findings which showed that cement did not cover all of the resorbed enamel surface.

Although the deposition of cement is followed by enamel resorption, enamel resorption may be an immunologically mediated event, similar to that noted to occur in the calcified tissues of deciduous brachyodont teeth during their shedding (Ten Cate 1994). Prior to cementogenesis, the reduced enamel epithelium disintegrates, exposing the enamel to the surrounding vascular dental sac. At this stage enamel is fully formed and is an inert, virtually dead tissue. This characteristic may induce an immunological reaction and odontoclasts, previously termed osteoclasts (Jones and Boyde 1974) gather around and resorb enamel. These odontoclasts are large multi-nucleated cells that result from merging of mononuclear cells of macrophage lineage (Ten Cate 1994). One purpose of cement deposition on the enamel surface may be to cover its surface and thus prevent further resorbtion by odontoclasts.

The current study showed that the cemental interface of enamel contained both macroscopic and microscopic irregularities, i.e. crests of perikyma (the transverse grooves and ridges on the surface of teeth enamel), resorptions bays and irregularities resulting from different rates of odontoclastic etching of prisms and interprismatic enamel. The crests of perikyma represent the macroscopic irregularities at the amelocemental junction of the buccal aspects of upper peripheral enamel, the infundibular enamel and the lingual aspects of the lower cheek teeth enamel. Irregularities due to resorption bays and different degrees of odontoclastic etching of prisms and interprimstic enamel have also been reported in equine coronal cement by Jones and Boyde (1974). However, in the current study, SEM examination of the surfaces of resorption bays did not reveal any irregularities resulting from different degrees of odontoclastic etching of crystals within the prism territories.

8. GENERAL CONCLUSIONS

The gross, histological and ultrastructural anatomy of equine enamel, dentine and cement have been described utilising a variety of specimen fixation and microscopic techniques and using different magnifications on a large number of specimens taken from a range of sites on the teeth. In addition to descriptions of the local anatomical structures, attempts were made to relate gross and subgross anatomy such as the variation in eq. enamel types at the different sites of the gross enamel folds. The relationship between the three different calcified dental tissues were also examined and finally, the relationship between structure and function was also considered, such as comparison of the degree of enamel prism decussation of incisor as compared to cheek tooth enamel. The findings of this study fully support Koenigswald and Clemens' (1992) assertion of the need to take an overview of the entire dental structure and not to concentrate on localised ultrastructural characteristics of a single dental tissue.

Having more fully defined the normal structure of the equine calcified dental tissues, studies on diseased tissues may now be more rationally pursued. Although this study was not primarily concerned with equine dental disease, some observations which may be of value to such pathological studies were made. The histology of "central infundibular cemental hypoplasia" and "junctional cemental hypoplasia" have been more fully described than hitherto. The description of central infundibular cemental hypoplasia where the defect only involved the apical aspects of the infundibulum and where the occlusal surface was normal does not appear to have been reported previously. These two cemental defects may be involved in aetiology of equine dental caries and merit further detailed consideration. The finding of a

relatively weak bond between enamel and cement may also have significance in the clinical condition of peripheral cheek teeth fractures that occur without underlying caries and this area also merits further work. Finally, this more complete description of the dental ultrastructure of *E caballus* will hopefully facilitate further taxonomic studies of equidae.

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